VOLUME 98

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NUMBER 2

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February, 1956

An Electronmicroscopic Study of the Fine Structure of Pulmonary Capillaries and Alveoli of the Mouse. Preliminary Report.
H. E. KARRER 65

Antibiotics in Experimental Tetanus: In Vitro and In Vivo Studies.
AHMAD ALI ANWAR AND THOMAS B. TURNER 85

Some Factors Influencing the Formation of L.E. Cells. A Method for Enhancing L.E. Cell Production.
WILLIAM H. ZINKHAM AND C. LOCKARD CONLEY 102

The Pathology and Pathogenesis of Experimental Anaphylactic Glomerulonephritis in Relation to Human Acute Glomerulonephritis.
ARNOLD R. RICH 120

Abstracts of Papers Presented Before the Meeting of the Johns Hopkins Medical Society. November 14, 1955 152

Book Reviews 154

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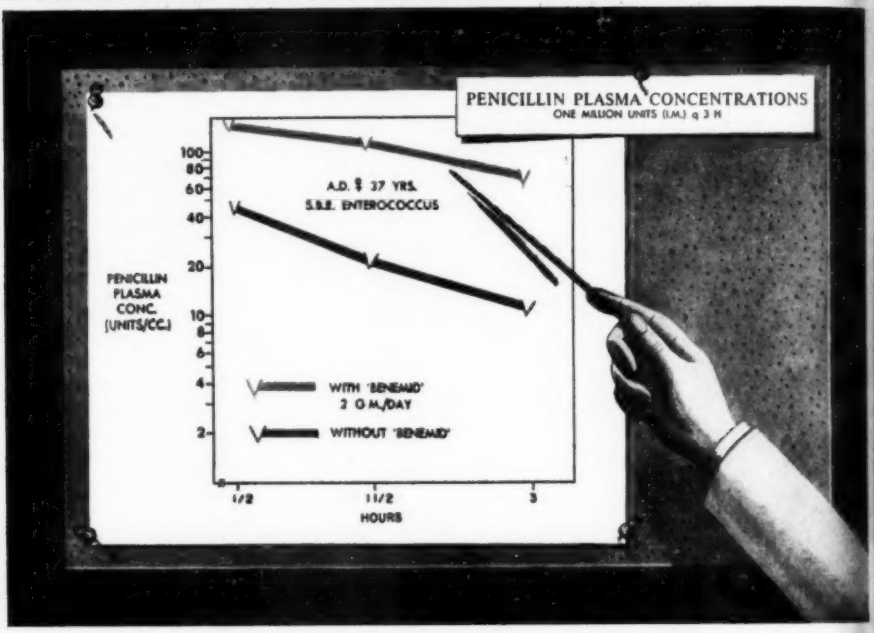

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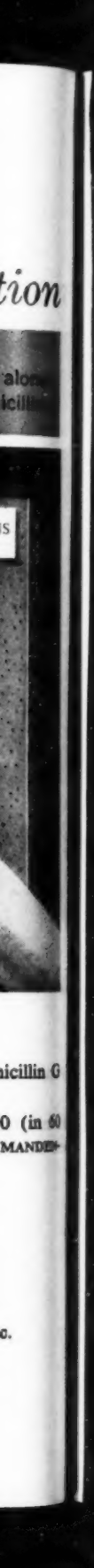

AN ELECTRONMICROSCOPIC STUDY OF THE FINE STRUCTURE OF PULMONARY CAPILLARIES AND ALVEOLI OF THE MOUSE

Preliminary Report

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Received for publication July 26, 1955

The fine structure of the alveolar wall has long been a subject of debate (1). Some histologists using light microscopic techniques have recently questioned the presence of an alveolar epithelium in the dog, rat, and mouse lung (2, 3). This is in contrast to most of the previous findings. Recently, on the basis of electronmicroscopic examination of the alveoli of rats, mice, rabbits, guinea pigs, dogs, and of man, Low reported the following (4, 5):

A. The presence of an alveolar epithelium in all the species studied.

B. Two parallel "basement membranes" underlying the alveolar epithelium and the capillary endothelium.

C. The presence of collagen and elastic fibrils in the alveolar wall.

A subsequent study (6) by Low deals with the fixation of lung tissue; short (15 minutes) osmium fixation was found to give the best results.

Swigart and Kane, using electron microscopy, have questioned the presence of the alveolar epithelial cells (7).

Clemens used thick (5 to 50μ) sections in the electron microscope. In thin areas of such sections he could see a membrane that covered the outside of the lung capillaries (8).

Policard and others described the capillary wall to be 0.15–0.25μ thick, but they could not see the capillary endothelium in many places (9). They believed that alveolar epithelial cells do not form a continuous layer.

Schlipköter (10) described the alveolar wall of rats as consisting of two layers; their thickness is given as 0.2–0.4μ and 0.1μ respectively. He showed electron micrographs of alveolar epithelial cells containing globular, concentrically lamellated inclusions amidst normal mitochondria.

Kisch states that "it seems difficult to doubt that very many capillaries in the lung are in direct contact with the air" (11). In his electron micrographs he shows "specific cells", characterized by numerous strongly osmiophilic inclusions.

METHODS

The mouse was the only species studied. Adult white mice (Princeton strain) of either sex were killed without anesthesia by breaking their necks. It was realized that this method

* Supported by a Grant-in-Aid from the National Cancer Institute, U. S. Public Health Service.

of killing could possibly cause aspiration of blood especially if the trachea was injured by the procedure, but it was nevertheless hoped that this method was the least traumatic for the lung tissue.

Fixation was performed as follows. About 0.2 cc. of buffered (12) 1 per cent osmium tetraoxide in isotonic saline was injected into the left pleural cavity immediately after the death of the mouse. The trachea was exposed and ligated just below the larynx; 0.5 cc. osmium tetraoxide was then injected into the trachea below the ligature, and the needle withdrawn immediately after injection. When the chest was opened, both lungs were already brown because of fixation, and were not collapsed. The anterior, posterior, and costodiaphragmatic margins of the left upper lobe were trimmed off *in situ*, fine scissors being used. One to two cc. of fixative were then injected into the right ventricle of the heart. When the vascular perfusion with fixative was successful, the fluid was observed as it appeared on the cut surfaces of the lobe. The left upper lobe was then removed, and parts of it were cut into pieces 1 mm. or less in diameter, two new razor blades being used. The specimens were then left in osmium fixative for two hours at 0° to 4° C.

After fixation, the pieces were washed for two hours in a large volume of distilled water in an Erlenmeyer flask immersed in ice water. The distilled water was renewed once after 1 hour. The flask was agitated from time to time during washing.

Dehydration was performed by immersion for periods of one half hour in 50, 80, and 3 changes of 100 per cent acetone.

Impregnation was carried out in a mixture of 4 volumes of butyl methacrylate and 1 volume of methyl methacrylate, containing one half per cent of either 2,4 dichlorobenzoyl-peroxide or of benzoylperoxide. Three changes of monomer were made during a total of at least two hours.

The tissues were embedded in prepolymerized methacrylate from the same batch as used for impregnation, in #5 gelatin capsules. An oven temperature of 80° C was used for polymerization, since E. Borysko has shown that at temperatures higher than 45° C tissues are better preserved (13)*.

A Servall microtome was used for sectioning (14), and an RCA EMU microscope for the microscopical study.

RESULTS

In lungs fixed as described above the alveoli were not collapsed, as seen in an optical photomicrograph of a stained thick section (Fig. 1). In the much smaller thin sections as used for electron microscopy only short fragments of alveolar septa were found.

In Figure 3 such a short fragment of an inter-alveolar septum is shown, which contains a capillary. The surface layer of the septum is formed by the alveolar epithelium. Such an epithelial layer has been seen in every one of numerous photographs. Below the epithelium there is a translucent space, which is limited by two dense membranes. The lumen of the capillary is lined with a monocellular sheet of capillary endothelium (Fig. 3).

Capillary endothelium

This layer in places exhibits typical nucleated endothelial cells (Figs. 5, 6); in other areas it becomes quite thin, down to a minimum of approximately

* Thankful acknowledgment is given to E. Borysko for his valuable advice regarding embedding, prior to publication of his findings.

100 Å (Figs. 11, 12). No discontinuity of this layer was observed. The border between two adjoining endothelial cells seems to be represented by a narrow space, which is in continuity with the space between the capillary endothelium and the alveolar epithelium, and also with the capillary lumen (Figs. 3, 4). This narrow space or cleft between two endothelial cells has a width of approximately 100 Å. Thus the single endothelial cell seems to be limited on all sides by a dense membrane; where two cells adjoin, their surface membranes run parallel and form the cleft-like space between them. Small mitochondria within the endothelium show interior double lamellae and an exterior membrane. The latter membrane rarely appears to be doubled (Figs. 3, 4, 6). Numerous inclusions in the form of small vacuoles are present everywhere in the cytoplasm; some of them seem to pinch off from the free surface (Figs. 4, 7). Rarely, large (0.8 to 2μ in diameter) intracellular vacuoles are seen, which cause a bulging of the cell surface into the capillary lumen (Figs. 3, 7). There is suggestive evidence for the presence of a structure comparable to the "endoplasmic reticulum" (15) (Figs. 5, 6), although further material must be surveyed in order to clarify this point.

"Basement membrane"

A narrow, structureless layer of translucent material lies between the alveolar epithelium and the capillary endothelium. It is limited on each side by a thin dense membrane (Figs. 3, 11, 12). The inner dense membrane can be considered to represent the surface membrane of the capillary endothelial cells; similarly the outer membrane could be interpreted as part of the alveolar epithelium. In some regions the space between the two membranes appears to be thickened (Fig. 8), perhaps because of oblique sectioning. It may be found dividing (Fig. 3). Its density is slightly greater than that of methacrylate and supporting formvar film (Figs. 8, 11, 12). Frequently, cloudy lighter areas are apparent within this substance (Fig. 8).

Alveolar epithelium

The alveolar air spaces are found to be lined by a thin uninterrupted layer with an average thickness of about 100–200 mμ (Figs. 2, 3, 11, 12), but may, in places, measure as little as 10 mμ (Fig. 8). Relatively infrequently alveolar epithelial cells (Figs. 9, 10) are seen bulging into the alveolar lumen. These nucleated cells seem to be continuous with and of the same fine structure as the thin epithelial layer lining the rest of the alveolus. The thin part of the alveolar epithelium has an inner structure consisting of small vacuoles and granules (Figs. 3, 11, 12); in some areas (Fig. 11) a vacuole seems to be forming from the surface. Mitochondria have been seen in the thin portions of this epithelium.

DISCUSSION

Alveolar epithelium

This report confirms the previous finding (4, 5) of a continuous thin alveolar epithelial layer. Although it has been found to be somewhat thicker than in Low's study of mouse lung, at its extreme thinness it is thinner than the 20 mμ given by Low. Its dimensions are in any case just at or below the resolution of the light microscope. The so-called "free cells", and interruptions in the epithelial layer (Low) were not found. The abrupt transition in the region of the nucleus from a bulging epithelial cell to a thin peripheral layer of cytoplasm is rather unusual. But it can easily be seen in sections of tissues cultured on glass surfaces (16) that living cells can attenuate to such extremely thin areas. Quite possibly the "septal cells" (2, 3) correspond to the bulging nucleated part of the epithelial cells; since other workers could not see the thin part of the epithelium in the optical microscope, they assumed the "septal cells" to be discontinuous. Small invaginations of the free surface (Fig. 11) may possibly be interpreted as volumes of fluid being ingested by the cell, i.e. as pinocytosis (17), since assumedly there is a fluid layer between the epithelial surface and the alveolar air.

The "specific cells" seen by Kisch (11) are really another type of alveolar epithelial cells as has been recognized by Schlipköter (10). We shall deal with them in another publication.

Capillary endothelium

The extreme thinness of the capillary endothelium is clearly demonstrated. In most areas this endothelium seems thinner than the alveolar epithelium, although measurements may be misleading because of the small sample of material surveyed. Oblique sectioning of the thin cells can also create the impression of an increase in thickness. In places there is suggestive evidence for pinocytosis (Figs. 4, 7). No attempt has been made to analyse the structure of the mitochondria inside the endothelial layer.

There are no pores in the endothelium of lung capillaries contrary to the findings in kidney capillaries (Pease (18), Rhodin (19), Bargmann (20)).

"*Basement membrane*"

A "membrana propria" or "basement membrane" can be seen as the underlying element of epithelial cells in general (20) and of capillary endothelia (21). It has been interpreted in the past as a product of either the epithelial cells or of the underlying connective tissue (20). It was seen to contain reticulin fibrils (21). In an electron microscopic study of kidney tubule epithelia (22) the underlying basement membrane has been described as a "complete sheath

around the tubule", of 0.05–0.1μ thickness, which was separated from the basal surface of the epithelial cells by a 200 Å wide space. Low (5) designates as "basement membrane" the electron-dense "basal surface of both (alveolar)* epithelium and (capillary)* endothelium".

In the present paper, the classical term "basement membrane" has been maintained. It is used to designate the space between capillary endothelium and alveolar epithelium. The electron-dense membrane that forms the base of the capillary endothelial cells obviously is nothing else but the surface membrane of these cells (Fig. 4). The second, parallel electron-dense membrane may analogously be interpreted as possibly representing the cytoplasmic membrane of the alveolar epithelial cells.

Most investigators of the past confirm the presence of a membrana propria or basement membrane in the wall of mammalian lung alveoli (20), and of reticulin, collagen, and elastic fibrils inside this membrane (23). The presented micrographs do not show any fibres in this layer, but in ultrathin sections they could easily be missed, or not be recognized. Moreover, Low has pointed out elastic and collagen fibrils in his electron micrographs (5), which lie in this region. This layer between endo- and epithelium is thus comparable to both the basement membrane known to underlie epithelial cells (20, 22) and to the capillary basement membrane (21). The term has therefore been maintained by us for purposes of convenience.

The "basement membrane" is continuous with intercellular layers between the single endothelial cells. Its composition cannot be determined from the electron micrographs. Cloudy light areas inside the membrane are most probably artefactual. Wider areas of up to 0.5μ (Fig. 8) are probably due to oblique sectioning. Wide separations of endo- and epithelium as observed by Low (5) have not been seen in the presented pictures. It could be assumed that separations of endo- and epithelium as observed in edematous frog lung (3) take place in the stratum of the "basement membrane".

SUMMARY

The structure of lung alveoli of adult mice has been investigated by means of the electron microscope.

The alveoli are lined by an uninterrupted thin epithelial layer consisting of cells.

The endothelium of the lung capillaries, also without discontinuities, reaches in places the extreme thinness of approximately 100 Å.

The classical term "basement membrane" has been maintained as designation for the layer between epithelium and endothelium. It appears as struc-

* Inserted by the author

tureless in the presented micrographs, but the presence of fibrils cannot be excluded. It is continuous with intercellular layers between single endothelial cells.

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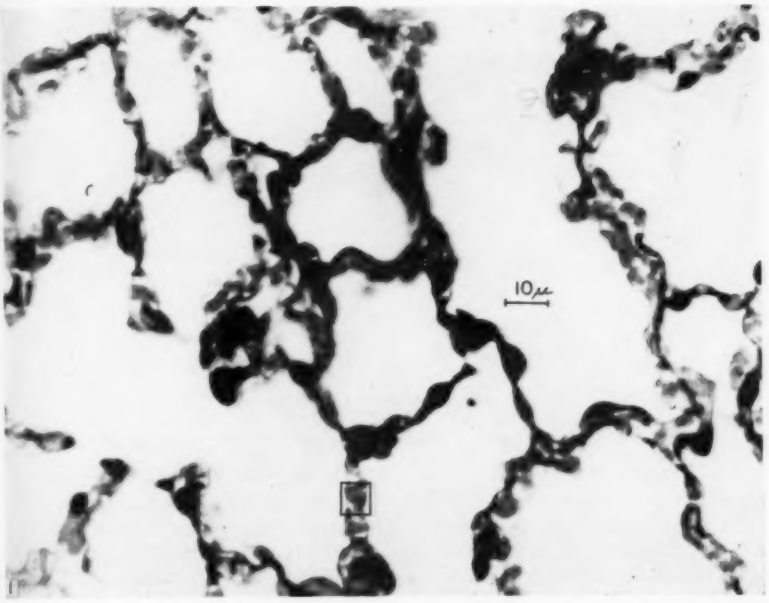
PLATE I

FIG. 1. Optical photomicrograph of thick section obtained from methacrylate-embedded specimen, stained with haematoxylin and eosin. The alveoli are not collapsed. Electron-micrographs like fig. 2 or 3 will show approximately as much tissue as is contained within the small rectangle. Magnification: 840 ×

FIG. 2. Capillary loop inside an interalveolar septum. Innermost is the capillary lumen containing a red cell. It is lined by the endothelium (ce), very thin at the right side of the photograph. An intercellular space is partially sectioned (isp). The outermost layer is the alveolar epithelium (ae), separated from the endothelium by an intercellular space (bm), the "basement membrane", which appears widened on the left side of the figure, perhaps because of oblique sectioning. Magnification: 29,300 ×



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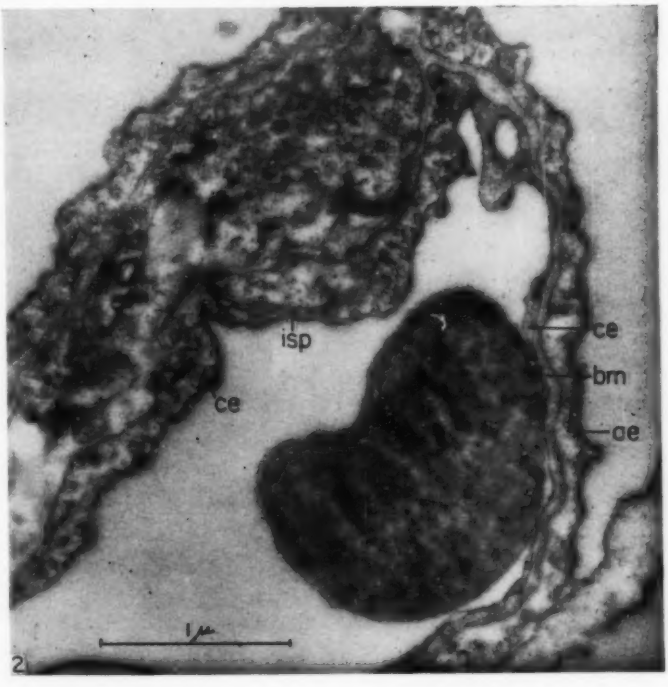

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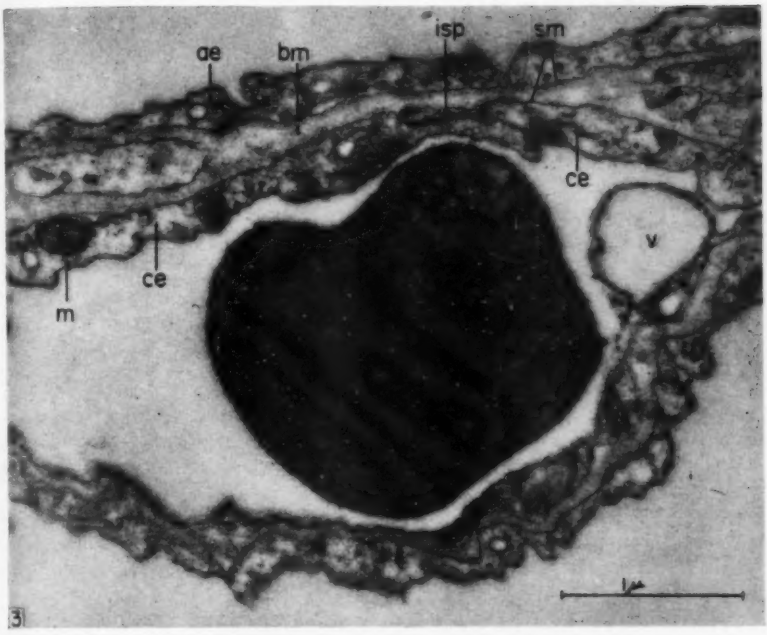
PLATE II

Interalveolar septum containing capillary. Survey and detail

FIGS. 3 and 4. A red cell is seen in the capillary lumen. An intercellular space (isp) between two capillary endothelial cells (ce) is in continuity with the "basement membrane" (bm). A large (v) and many small vacuoles and mitochondria (m) are visualized inside the endothelial cytoplasm. The continuous alveolar epithelium (ae) represents the outermost layer on both sides of the interalveolar septum. The "basement membrane" (bm) between the two cellular layers is confined between two electron-dense membranes (sm) which may be considered to be the surface membranes of capillary endothelium and alveolar epithelium. At (p) possible pinocytosis. Magnification: Fig. 3, 29,000 ×; fig. 4, 68,000 ×



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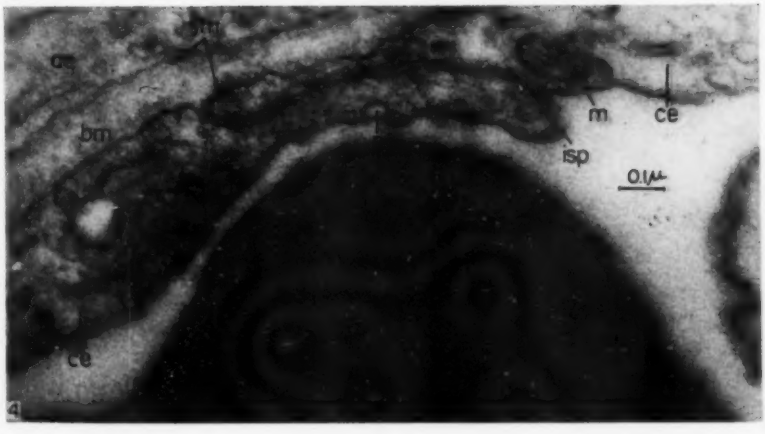

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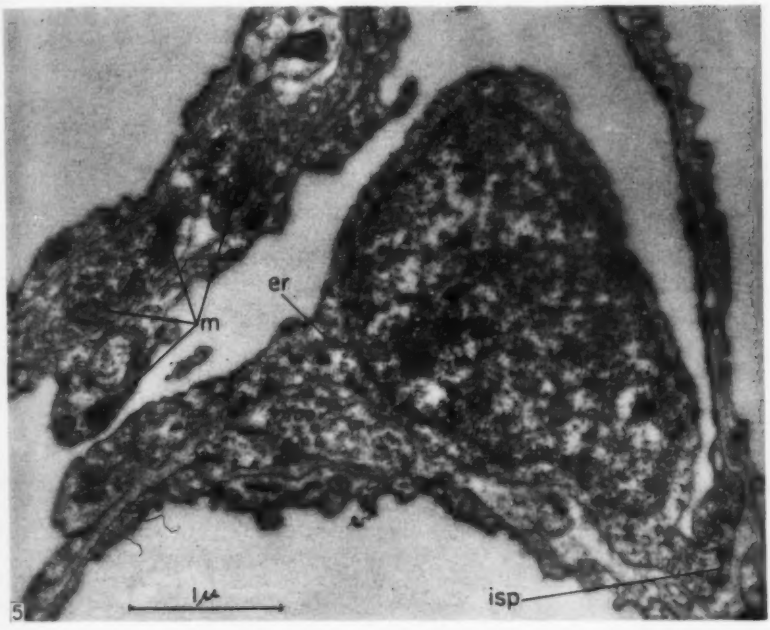
PLATE III

Capillary endothelial cells

FIG. 5. The endothelial cell (with nucleus) is bulging into the capillary lumen. In the right lower corner of the figure an intercellular space (isp) (compare figures 3 and 4). "Endoplasmic reticulum" inside the endothelial cell at (er), mitochondria at (m). Magnification: 23,000 ×

FIG. 6. The endothelial cell (with nucleus) is seen on the left; at right a leukocyte within the capillary (with terminal section through the nucleus, and numerous mitochondria). "Endoplasmic reticulum" (er) and mitochondria (m) inside the endothelial cell. Magnification: 18,200 ×



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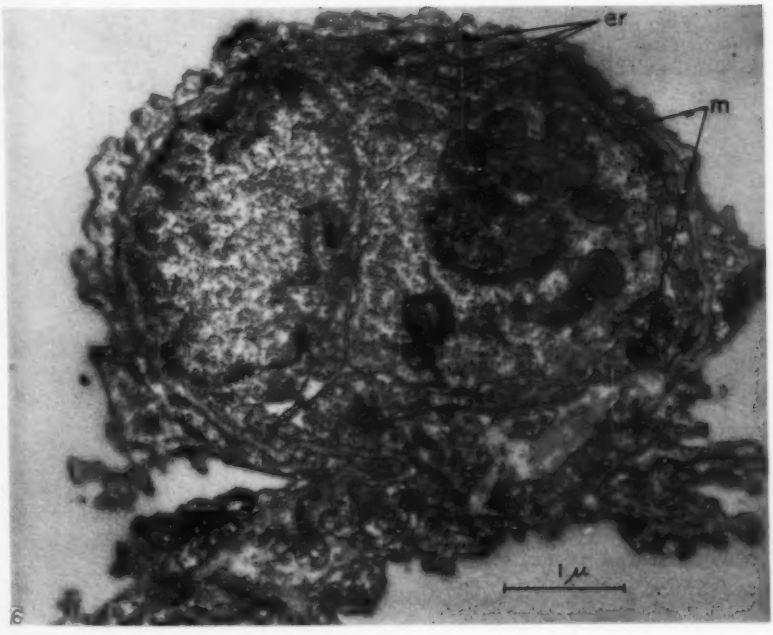

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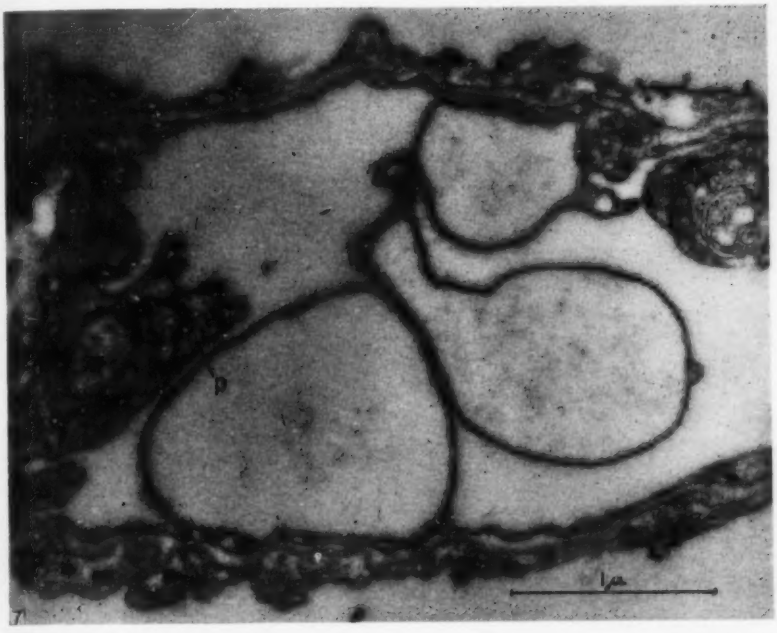
PLATE IV

FIG. 7. Large vacuoles inside the capillary endothelium, which in most places is very thin. Suggestion of pinocytosis by the endothelium (p). Magnification: 31,000 ×

FIG. 8. Section, presumably oblique, through endothelium (ce), showing vacuole-like and granular structures in the cytoplasm. In one area the alveolar epithelium is approximately 100 Å thick (ae). The "basement membrane" (bm) between the two cellular layers is widened, presumably because of oblique sectioning. Magnification: 77,000 ×



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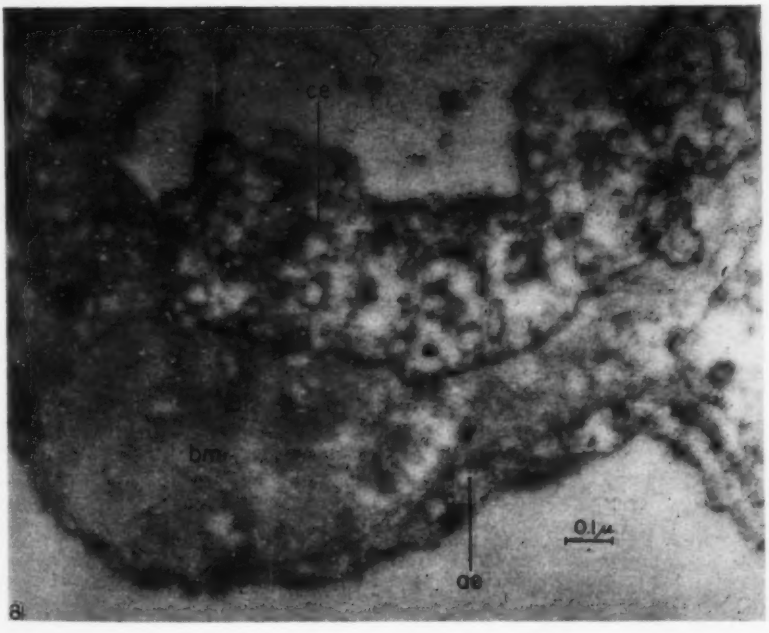

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PLATE V

Alveolar epithelial cell. Survey and detail

FIG. 9. An interalveolar septum is running from left to right; alveolar lumen at top and bottom of the picture. Inside the septum a capillary contains three red cells, and (left) one white cell. The thin alveolar epithelial layer (ae) that lines the alveolus at top of the picture is seen to thicken to a multiple of its width (at right from the center of the photograph) and to contain a nucleus. Magnification: 8,800 ×

FIG. 10. There is an uninterrupted transition from the bulging nucleated part of the alveolar epithelial cell into the thin part (ae) of the alveolar epithelium. Magnification: 13,300 ×

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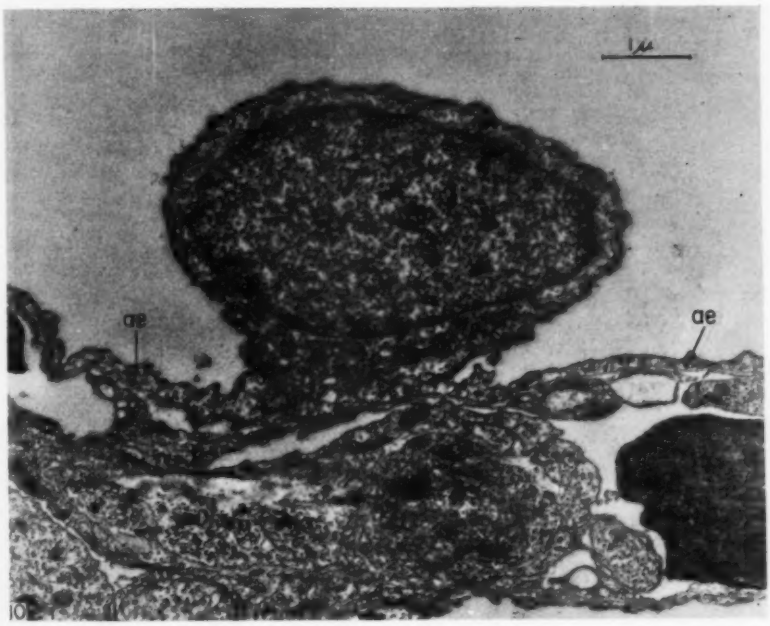

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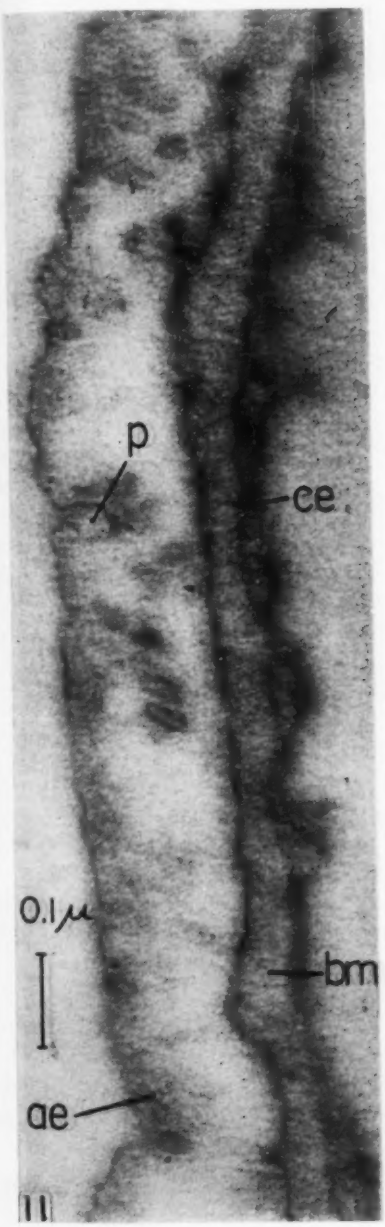
PLATE VI

The "blood-air-barrier". Capillary endothelium (ce), alveolar epithelium (ae), and "basement membrane" (bm).

FIG. 11 (left). At (p), indication for pinocytosis by the alveolar epithelium. Magnification: 146,000 ×

FIG. 12 (right). At (x) the capillary endothelium has an overall thickness of approximately 100 Å (including both electrondense surface membranes). Note the several very thin areas in the endothelium, which with less resolution would possibly be interpreted mistakenly as holes in the endothelium. At (isp) there is probably the border between two endothelial cells (compare figures 3 and 4). Magnification: 97,000 ×



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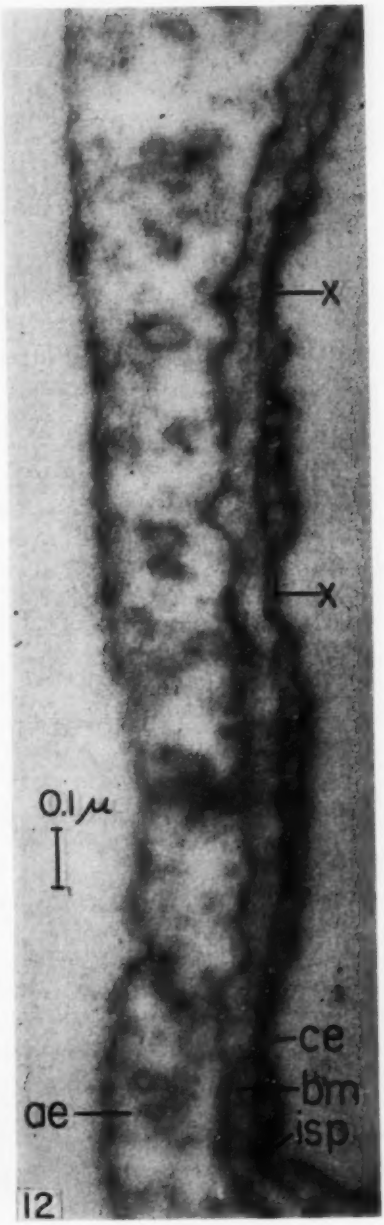

12

ANTIBIOTICS IN EXPERIMENTAL TETANUS: IN VITRO AND IN VIVO STUDIES[1]

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The Department of Microbiology, The Johns Hopkins University

Received for publication September 27, 1955

Previous studies (1–6) have given some indications of the effectiveness of antibiotics against *Clostridium tetani* in vitro and in the experimental disease in animals, but on the whole published comparative data are meager. Likewise, the difficulty in making controlled observations on either the prophylactic or therapeutic value of these drugs in tetanus in man leaves the limits of their usefulness ill defined, to say the least. The present experiments were designed to define some of the areas of effectiveness of present day antibiotics in experimental tetanus in the expectation that such data might provide a rational basis for their use in man.

In one of the earliest papers on penicillin, Abraham *et al.* (1) made a passing reference to the inhibitory effect of this antibiotic on *Cl. tetani* in vitro. Kiser *et al.* (2) found that *in vitro* growth of this organism in thioglycollate broth cultures was inhibited by 0.63 micrograms of terramycin (oxytetracycline) per ml.; 0.16 μgm. of aureomycin (chlorotetracycline); 2.5 μgm. of chloroamphenical; and 0.02 μgm. of penicillin.

Inhibitory concentrations of various antibiotics for *Cl. tetani* in broth cultures as determined by English *et al.* (3) were oxytetracycline 0.31 μgm. per ml., chlorotetracycline 0.03 μgm., and tetracycline 0.31 μgm. Lund (4) also found that germinating tetanus spores were sensitive to penicillin. A criticism of all these *in vitro* studies might be that apparently a standardized inoculum of a known number of viable organisms or spores was not used.

Working with experimental tetanus in mice, Novak, Goldin, and Taylor (5) found that penicillin G in sesame oil and 2 per cent aluminum monosterate protected a high proportion of mice when administration of the antibiotic was begun 24 hours after the injection of washed tetanus spores, but was less effective when administration of penicillin was delayed beyond 24 hours. Bliss, Wrath, and Chandler (6) in somewhat similar experiments found that when administered at six-hourly intervals beginning immediately after the infecting dose of tetanus organisms, terramycin and penicillin gave better protection to mice than did aureomycin. Kiser *et al.* (2), however, observed that aureomycin in 5 daily doses of 20 milligrams per kilogram of body weight of mice gave good protection, and 10 mgm./kg. partial protection.

[1] These studies were supported by a grant from the Whitehall Foundation, Inc., New York.

[2] Presently Associate Professor of Microbiology, University of Karachi, Pakistan.

Reported results of the use of antibiotics in clinical tetanus of man are difficult to evaluate. Weinstein and Wesselhoeft (7) observed two cases of severe tetanus treated with large doses of penicillin with what they regarded as favorable results; *Cl. tetani* could not be cultured from the wounds 24 hours after surgical treatment of the wounds and administration of penicillin. Buxton and Kurman (8) reported two cases of tetanus in which penicillin was used; Altmeir (9), 16 cases; and Dias-Rivera, Deliz and Berio-Saurez (10), 59 cases. Altmeir could find no evidence that penicillin exerted a favorable effect on the outcome, while Dias-Rivera *et al.* concluded that penicillin was a useful adjunct in the treatment of the disease. Obviously it is extremely difficult to observe anything approaching a controlled series of clinical cases, and consequently to obtain convincing data on the effect of one or another antibiotic from a study of the disease in man.

MATERIALS AND METHODS

Four different strains of *Cl. tetani* were tested for their ability to produce toxin on artificial media, and the best of these, Strain t_{1-1} obtained through the courtesy of Dr. John Brewer of the Hynson, Westcott and Dunning Co., was used throughout this study. In all the *in vitro* and *in vivo* experiments in this study, washed spore suspensions of known viable counts were used.

The spore suspensions were prepared as follows: From a stock culture tube of cooked meat medium, a loopful of inoculum was introduced asceptically into a flask of veal infusion broth[3] which supports good toxin production as well as good growth of organisms. The organisms were incubated in this medium for 7 days at 37°C. and anaerobic agar plates were inoculated from this flask. The plates were in turn incubated for 7 days at 37°C. and the growth washed with sterile distilled water, shaken with sterile glass beads to break up the clumps of organism, and filtered through a thin layer of cheese cloth and cotton. This suspension of organisms was then heated in an 80°C. ±1° water bath for 20 minutes in order to kill the vegetative forms and inactivate any toxin that might be present. The spores were washed three times with physiologic salt solution followed by centrifugation each time. The final centrifugate was then suspended in 50 ml. of saline to form a stock suspension. The supernate after the last centrifugation was tested in mice for the presence of tetanus toxin, always with negative results.

Preparation of spore suspensions: Serial dilutions were made from the stock suspension and viable spore counts obtained. One ml. of spore suspension of each of the tenfold dilutions from 10^{-2} to 10^{-6} was pipetted into each of two small Erlenmeyer flasks containing 15 ml. of melted anaerobic agar. The contents of each flask were mixed and poured into a sterile deep petri plate. The agar was allowed to harden and then a second layer of 35 ml. of agar was poured over the first one. The plates were incubated at 37°C. for 48 hours and the number of colonies counted. This counting procedure was carried out twice on each batch of spores.

Preparation of antibiotic suspension: The following antibiotics were tested in the study: terramycin, aureomycin, achromycin, chloromycetin, ilotycin, magnamycin, streptomycin, and penicillin G, potassium salt. All antibiotic products were obtained through regular com-

[3] Veal infusion 500 grams. Proteose Peptone 10 grams. Bacto Agar 1 gram. Distilled water 1000 ml.

mercial sources. The injectable forms of the drugs were dissolved either in sterile distilled water or in saline as indicated on the vials. The powdered or tabloid forms of most of the oral drugs were dissolved in water with the aid of hydrochloric acid as described by English *et al.* (3), and pH adjusted to 8.3 with sodium glycinate buffer. Subsequent dilutions were generally made in thioglycollate broth for *in vitro* studies and in saline for *in vivo* injections.

Methods of in vitro testing: Tests of antibiotics *in vitro* were made in thioglycollate broth. Different concentrations of each antibiotic were obtained in a final volume of 10 ml. of thioglycollate broth. All tubes, including the controls to which no antibiotics were added, were inoculated with 0.1 ml. of a spore suspension of *Cl. tetani* containing commonly approximately 100 viable spores; exceptionally 1000 spores were inoculated. The tubes were incubated at 37°C. and final readings for visible growth taken at the end of 72 hours.

Method of in vivo testing: Spore suspensions prepared by the method given above were tested initially for pathogenicity in mice. Mice weighing 18 to 20 grams were inoculated with 0.2 ml. of 10^{-2} spore suspension (containing approximately 1000 viable spores) and 3.5 to 4.5 per cent solution of calcium chloride. The spore suspension and calcium chloride were mixed in equal amounts and injected subcutaneously at the base of the tail of each mouse. With the first batch of spores, which was used throughout most of this study, it was observed that 80 to 90 per cent of mice infected by this method died of tetanus. However, a later batch, which was used mostly in studies with rabbits, caused death in 100 per cent of mice. In preliminary studies it was consistently observed that, if *Cl. tetani* is grown in a medium favorable for toxin production, and this source is used for inoculating Brewer's anaerobic plates for spore production, then the pathogenicity of spores is significantly greater than if thioglycollate broth culture is used as a source of inoculum. The above dose of inoculum was employed for all *in vivo* tests in mice. The inoculum used for hamsters and guinea pigs was about 5000 viable spores, and for rabbits 10,000. However, in the final experiments in rabbits the inoculum used contained 40,000 viable spores. Hamsters, guinea pigs and rabbits were inoculated intramuscularly in one hind thigh.

Course of infection in experimental animals: Mice: In mice the period before the first signs of tetanus appeared was about 72 hours, when inoculated subcutaneously with the dosage of spores used. The animals first showed signs of stiffening and partial paralysis of the hind leg on the inoculated side. The disease then progressed rapidly with curling of the tail towards the inoculated side, and outward curvature of the body trunk due to contraction of the abdominal muscles on the injected side. The animal became spastic and enfeebled and died within 24 to 36 hours after the appearance of symptoms.

Hamsters: Hamsters showed about the same incubation period following inoculation of spores as the mice, and the general course of the disease was essentially the same. However, the time from the onset of disease to death in hamsters was relatively short, the animals usually dying within 18 to 24 hours after onset of disease.

Guinea pigs and rabbits: The course of the disease and the symptomatology in guinea pigs and rabbits, inoculated with the spores of *Cl. tetani*, were similar. The incubation period, with the dosage of spores used, varied somewhat from animal to animal, with the first symptoms usually appearing about 48 to 60 hours after introduction of spores. The first sign of the disease was stiffening of the inoculated leg which tended to remain in extension as the disease progressed. In most untreated animals, the disease progressed rapidly into generalized tetanus, with marked opisthotonus, clonic spasms, convulsions when disturbed, and death within 48 hours after onset of first signs.

Antibiotic treatment: The dilutions of antibiotics were prepared as detailed above. The antibiotics were administered intraperitoneally in mice and hamsters and intramuscularly in rabbits. Unless otherwise indicated, the treatment was begun within an hour after inocu-

lation of spores and five doses, at daily intervals, were given. All antibiotics were used on a milligram per kilogram of body weight basis. Inoculated but untreated animals served as controls. When the treated groups of animals were given antibiotics a corresponding amount of normal saline was injected into the control animals. In most cases mice and hamsters were retained for four to six weeks after the discontinuance of treatment and observed for the development of any late signs of tetanus. However, relapses later than two weeks after inoculations were not observed.

Antitoxin treatment: In experiments where comparative study of tetanus antitoxin was made, tetanus antitoxin, prepared in horses, was first titrated in mice to determine its actual strength in units, and diluted in infusion broth to the required unitage per ml. The method of administration of antitoxin into animals was similar to that of antibiotics, intraperitoneally in mice and hamsters, intramuscularly in rabbits.

RESULTS

In vitro studies: The results of the effect of antibiotics on *Cl. tetani in vitro* are given in Table I. Most antibiotics were tested in three different experiments and the results presented represent the range of sensitivity of the organism, and also the mean inhibitory concentration of the antibiotic in micrograms per ml.

It is evident from these results that the inhibitory range of antibiotics varied from drug to drug. The inhibitory range of the tetracycline group of antibiotics (aureomycin, achromycin, and terramycin) fell within the limits of fourfold dilutions, achromycin being a little more effective than the other two. Among the other broad spectrum antibiotics ilotycin and chloromycetin were also effective within the general range of the tetracycline group. However, magnamycin and streptomycin were relatively ineffective. Penicillin consistently inhibited growth at 0.6 micrograms or 1 unit per ml.

In order to determine the effect of a tenfold increase in the amount of inoculum (spores) on the inhibitory action of antibiotics, similar experiments using an inoculum of 1000 spores, instead of 100, were carried out. The results are

TABLE I

In Vitro Sensitivity of Clostridium tetani *to Various Antibiotics*

Antibiotic	Lowest Inhibitory Concentration of Antibiotic in Micrograms per Ml. with Original Inoculum of			
	100 viable spores		1000 viable spores	
	Range	Mean	Range	Mean
Achromycin	0.03–0.06	0.045	—	—
Ilotycin	0.065–0.125	0.095	—	—
Terramycin	0.16–0.16	0.16	0.31–0.62	0.47
Aureomycin	0.16–0.32	0.24	1.25–2.5	1.84
Chloromycetin	0.32–0.62	0.47	0.62–0.62	0.62
Penicillin	0.6–0.6	0.6	0.6–1.2	0.9
Streptomycin	5.0–10.0	7.5	—	—
Magnamycin	10	10	—	—

given in Table I. It is evident that when the number of viable spores was increased tenfold, from 100 to 1000, the amount of antibiotics needed to inhibit growth was increased generally by two to four times. However, in the case of aureomycin an eightfold increase was required.

Mode of action of antibiotics on Cl. tetani in vitro: In the limited number of reports on the effect of antibiotics on *Cl. tetani* the action of the drug has been referred to as "inhibitory" or "effective". These terms did not actually indicate whether the antibiotics used were bactericidal or bacteriostatic. Furthermore, most of these reported results were inconclusive with respect to the mode of action of these drugs and as to whether the action was on bacilli or on spores of *Cl. tetani.*

Currans and Evans (11) reported that penicillin in low concentration (5 units per ml.) was lethal to the spores of 5 different aerobic species. Lund (4) showed that germinating spores of *Cl. tetani* were sensitive to 1 unit of penicillin, whereas the resting spores were resistant to more than 10,000 units of penicillin. In his studies Lund did not report quantitative determinations with regard to the number of viable spores before and after exposure to the antibiotics.

In an effort to determine whether the effect of antibiotics in low concentrations was bactericidal or bacteriostatic the following experiment was done. Thioglycollate broth tubes containing the lowest inhibitory dilutions (determined by previous *in vitro* experiments) of aureomycin, achromycin, terramycin, ilotycin and penicillin were inoculated with 100 viable spores of *Cl. tetani* and incubated at 37°C. for seven days. There was no visible growth in any of these tubes. At the end of this period the contents of each tube were distributed in equal amounts among ten tubes containing 10 ml. of fresh thioglycollate broth. This procedure resulted in a tenfold dilution of any remaining antibiotic below the inhibitory threshold and provided a favorable environment for any viable bacilli or spores to resume growth. However, none of these sub-inoculated tubes showed visible growth even after a period of a week of incubation at 37°C. This experiment was repeated several times and consistently gave the same results, thus strongly suggesting that the mode of action of the antibiotics was bactericidal rather than bacteriostatic.

Action of antibiotics on spores

While the foregoing type of experiment showed clearly that certain antibiotics had a bactericidal effect, it did not indicate whether the action was primarily on the spore or on the vegetative form of the organism. This question was studied in the following experiment.

Heavy aqueous suspensions of washed spores (approximately 2 million viable spores per ml.) of *Cl. tetani* were mixed with equal volumes of the appropriate dilution of achromycin, aureomycin, ilotycin and terramycin, respectively, in

each tube, giving a final concentration of 25 μgm. per ml. for each antibiotic. Penicillin dilutions were mixed to give final concentrations of 100,000 units to 1 unit in a tenfold serial dilution system. Two sets of tubes were prepared for each dilution of antibiotic. These tubes were then incubated, one set at 4°C. and the other at 37°C. for seven days. At the end of this period the spores were washed with sterile water by alternate centrifugation and discarding of supernate. The final sediments of spores were suspended in 10 ml. of sterile distilled water.

To the dilution tubes of penicillin, 10 units of penicillinase were added, in order to remove any effect of penicillin that might still have been present after the four washings with water. Dilutions of 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} were prepared from each of the treated spore suspensions. Similar dilutions from the stock spore suspension stored at 0°C. served as control. One ml. of each of the dilutions were plated out with anaerobic agar, as detailed previously, for viable spore counts. The viable spore counts were obtained by counting the number of colonies on each plate at the end of 48 hours incubation at 37°C.

The results showing the effect of antibiotics on viable spore counts obtained by plate dilution technique are given in Table II. It is evident from these results that at 4°C. none of the five antibiotics tested had any action on the spores of *Cl. tetani*. However, at 37°C. in penicillin at concentrations of 100,000 units there were no viable spores left in a suspension originally containing 2 million spores per ml. Even at a dilution of 1000 and 100 units per ml. there was a reduction of 50 per cent in the viable counts of spores.

TABLE II

Sporicidal Action of Antibiotics on Clostridium tetani *Spores in Aqueous Suspension*

Antibiotic Conc. per ml.	Approximate Viable Spore Count in Millions after 7 days Incubation with Antibiotic	
	37°C. incubation	4°C. incubation
Achromycin, 25 μgm.	1.3	1.9
Aureomycin, 25 μgm.	1.6	2.6‡
Ilotycin, 25 μgm.	1.3	2.0
Terramycin, 25 μgm.	1.3	2.0
Penicillin, 100,000 U*	0	1.8
Penicillin, 10,000 U.	0.0001	2.0
Penicillin, 1,000 U.	0.032	2.1
Penicillin, 100 U.	0.9	3.0‡
Penicillin, 10 U.	1.0	2.2
Penicillin, 1 U.	1.2	1.9
Control—no. antibiotic	1.4	1.9
Control—original culture	2.2†	—

* Approximately 60 mgm.

† Before incubation.

‡ Count less accurate due to contamination of one dilution.

The above results strongly suggest a sporicidal action of penicillin at 100 to 100,000 units per ml. at a temperature of 37°C. At the later dilution complete destruction of spores seems to occur.

The interpretation of these results is based on the assumption that spores ordinarily will not germinate in saline suspensions alone in the absence of nutrients. This assumption is further justified by the observation that the other antibiotics, which seemed to have little or no effect under these conditions, would have been expected to be highly effective if vegetative cells were present, on the basis of the data presented in Table I. Taking these two sets of data together it would seem that the tetracycline group of antibiotics is effective only after germination of the spores, while penicillin is effective in high concentration against tetanus organisms in the spore stage. It is recognized that the evidence on these points is of an indirect nature.

Antibiotics in experimental tetanus

Results in mice: The effect of antibiotics in experimental tetanus was tested in mice, hamsters and rabbits. However, more extensive work was done in mice and confirmatory results obtained from the experiments in hamsters and rabbits. The detailed results from experiments in mice are presented in Table III. In the analysis and tabulation of results the Reed-Muench method of computation was used. The percentage deaths are reported as the per cent of cumulative deaths of the total. The protective dose (PD_{50}) represents the dose of the drug in mgm. per kg. body weight that permitted survival of 50 per cent of the mice. It should be noted that only 83 of 99 untreated control mice died, but no correction has been introduced to cover this fact. Ninety-five per cent confidence limits are given.

From these results it is apparent that terramycin, with a PD_{50} of 1.75 mgm. per kg. and confidence limits of 1.15 to 2.65, was the most effective of all the drugs tested. Among the remaining 4 drugs, penicillin with a PD_{50} of 8.1 seems relatively better than the others. However, there is not a significant difference in their relative effectiveness, as the range of their confidence limits over-lap each other.

Results in hamsters: Confirmatory results with regard to the relative effectiveness of penicillin, terramycin and aureomycin were obtained in hamsters. Seventy-four hamsters were inoculated with tetanus spores, as detailed above, and were then divided into eight groups of eight hamsters each and a ninth group of 10 hamsters, which served as controls. Three groups were then treated with terramycin in daily doses of 20, 10, and 5 mgm. per kg. body weight for 5 days, the first dose being given immediately after the inoculation of spores. Two groups were given aureomycin in daily doses of 20 and 10 mgm. per kg., respectively, for 5 days. Three groups were given penicillin in aqueous solution

TABLE III

Antibiotics in Experimental Tetanus of Mice. Inoculum Approximately 1000 Viable Spores per Mouse, 10 Mice per Treatment Group

Antibiotic	Daily Dose for 5 Days	Reed-Muench Cumulative Deaths	PD_{50} Dose*	Confidence Limits†
	mgm./kg.		*mgm./kg.*	
Terramycin	2.5	4	1.4	.9–2.3
	1.25	9		
	0.625	17		
	0.312	27		
Penicillin G	7.5	6	8.1	4.7–13.9
	3.75	14		
	1.87	24		
	0.98	32		
Aureomycin	40	3	10.6	6.0–18.5
	20	6		
	10	12		
	5	20		
	2.5	29		
	1.25	35		
Ilotycin	20	3	11.6	7.0–19.2
	10	10		
	5	16		
Achromycin	40	0	14.1	11.8–16.6
	20	2		
	10	10		
	5	20		
Controls—saline only	—	83/99	—	—

* PD_{50} = Dose which permitted survival of 50 per cent of mice.

† PD_{50} is the antilog of $\log PD_{50}$ determined by the Reed-Muench method, using a log scale of dose. $SE_{\log PD_{50}}$ was computed by Pizzi's formula (16, 17). The confidence limits are the antilogs of $\log PD_{50} - 2SE$ and $\log PD_{50} + 2SE$. They represent the uncertainty with regard to the "true" PD_{50}, as distinguished from the value obtained from our series of mice. There is a 95 per cent probability that the confidence limits embrace the PD_{50} which would be obtained from an indefinitely great series of mice.

in daily doses of 80, 40 and 20 mgm. per kg., respectively for 5 days. The control group of 10 hamsters was given 1 ml. of isotonic saline solution daily for 5 days.

In Table IV are shown the deaths occurring within a ten-day period with each scheme of treatment. Terramycin protected all the animals at the dosage levels employed. Penicillin gave protection to all the animals at the daily dosage level of 80 mgm., but to only about three-fourths to one-half of those treated with 40 and 20 mgm. per kg. The range of protection with the doses of aureomycin employed was of the same order of magnitude as with penicillin.

TABLE IV

Antibiotics in Experimental Tetanus of Hamsters: Inoculum Approximately 5000 Viable Spores

Antibiotic	Daily Dose for 5 Days	Number Inoculated	Deaths from Tetanus
	mgm./kg.		
Terramycin	20	8	0
	10	8	0
	5	8	0
Penicillin	80	8	0
	40	8	2
	20	8	3
Aureomycin	20	8	3
	10	8	4
Controls	None	10	7

Results in rabbits: Each of 13 rabbits was inoculated in the right thigh muscle with approximately 40,000 viable tetanus spores. Three rabbits were treated with 300,000 units (approximately 50–60 mgm./kg.) of procaine penicillin in oil, injected into the left thigh muscles immediately after the inoculation of spores, and a second dose of 300,000 units 48 hours later. Two rabbits were given only one injection of 300,000 units of procaine penicillin, this injection being immediately after the inoculation of spores.

Two rabbits were given two daily doses of terramycin in 20 mgm. per kg. body weight beginning immediately after inoculation of spores, and two animals received a total of 4 daily doses. Four rabbits which served as controls were given no treatment.

The results of this experiment, which are shown in Table V, not only confirm the results obtained in the studies with mice and hamsters, but also indicate the relative efficacy of the two drugs in eliminating infection with *Cl. tetani*. The control rabbits, developed rapidly progressive generalized tetanus on the third and fourth days after infection and died within the following 24 hours. By contrast, the four animals treated with terramycin remained without symptoms during the entire observation period of four weeks. Additional data on the effect of terramycin are given in a succeeding section. Penicillin gave a degree of protection, in that the two animals which received one dose only remained without symptoms for 4 and 5 days, respectively, but then succumbed to generalized tetanus within the succeeding 24 hours. Of the three animals given two doses of penicillin 48 hours apart, one remained without symptoms during the entire observation period of 28 days, while the other two developed generalized tetanus on the seventh and tenth days, respectively, and succumbed a few hours later.

TABLE V

Effect of Terramycin and Procaine Penicillin in the Prevention of Experimental Tetanus in Rabbits. First Dose Immediately After Injection of Tetanus Spores

Rabbit No.	Treatment		Signs of Tetanus Days after Infection	
	Antibiotic	Amount per dose and number of doses	First symptom	Death
54–38	Terramycin	20 mgm./kg.—4 daily doses	None*	—
54–69	Terramycin		None*	—
54–36	Terramycin	20 mgm./kg.—2 daily doses	None*	—
54–37	Terramycin		None*	—
54–26	Procaine penicillin	300,000 units†—2 doses 48 hours apart	None	—
54–28	Procaine penicillin		7	8
54–29	Procaine penicillin		10	11
53–65	Procaine penicillin	300,000 units—one injection only	5	6
53–66	Procaine penicillin		4	5
53–67	None	—	3	3
53–68	None	—	3	4
53–71	None	—	4	4
53–73	None	—	4	4

* Animal remained without signs of tetanus during observation period of 28 days.

† 300,000 units of penicillin represented approximately 50 to 60 mgm./kg. in these animals.

Studies on the mode of action of antibiotics and tetanus antitoxin in vivo

Data presented in a preceding section indicated that both terramycin and penicillin when tested *in vitro* were principally effective on the vegetative forms of *Cl. tetani*, and that except with very large amounts of penicillin, there was little or no lethal effect on the organism in the spore stage. The experiments presented in the present section were designed to extend the foregoing data to the mode of action of certain antibiotics and tetanus antitoxin in the intact animal.

Studies in mice: Mice were inoculated with tetanus spores as outlined previously, and groups treated with terramycin, penicillin, and tetanus antitoxin at 0, 24 and 48 hours post-inoculation. Each course of treatment consisted of 5 daily doses. The individual dose of penicillin G was 60 mgm. per kg. in aqueous solution; of terramycin 20 mgm. per kg.; of tetanus antitoxin approximately 2.0 units of a horse serum preparation per mouse.

The results of these experiments are presented in Table VI. In accordance with results obtained previously, terramycin was highly effective when given immediately after infection, and penicillin in a dosage of 60 mgm. per kg. was partially effective. Neither antibiotic, however, gave protection when their administration was delayed until 24 hours after infection. This result may have been due to the rather overwhelming infection induced, since most of the con-

TABLE VI

Comparative Effect of Antibiotics and Antitoxin Administered to Mice at Various Intervals after Injection of Tetanus Spores

Type of Treatment	First Treatment, Hours after Infection	Number of Mice in Group	No. Died of Tetanus
Terramycin 20 mgm./kg. each dose, five daily doses	0	10	0
	24	10	10
	48	10	10
Penicillin-aqueous, 60 mgm./kg. each dose, five daily doses	0	10	5
	24	10	10
	48	10	10
Tetanus antitoxin, 1 dose 2 units per mouse	0	10	8*
	24	10	5*
	48	10	10
Controls, no treatment	—	23	23

* Death delayed until ninth and tenth day.

trol mice were dead by the third day as were most of those in which the beginning of treatment was delayed until 24 or 48 hours. It will be noted in subsequent experiments in rabbits that terramycin treatment begun 24 hours after infection was effective.

The results with tetanus antitoxin were quite interesting in that all mice given antitoxin immediately after infection with spores remained symptomless until the ninth day, at which time typical signs of tetanus began to appear; by the tenth day 8 of the 10 mice were dead. Among the mice in which antitoxin was begun 24 hours after infection most of the deaths occurred in the first 4 days, while of those in which treatment was started at 48 hours all were dead by the fourth day. The foregoing results indicate that terramycin and to a lesser degree penicillin in the doses used were capable of destroying *Cl. tetani* in mice. When, however, administration of these drugs is delayed until substantial amounts of toxin have been produced and apparently fixed to the central nervous system, symptoms of tetanus will ensue, regardless of the action of the antibiotics on the bacteria themselves.

Studies in rabbits: Four groups of three rabbits each were inoculated intramuscularly in the right thigh muscles with approximately 40,000 viable spores in 4.5 per cent calcium chloride. Twenty-four hours later, one group was treated with terramycin, another group with terramycin and tetanus antitoxin, and a third group with antitoxin only; the fourth group received no treatment.

Surviving animals were observed for 28 days. Treatment with terramycin consisted on 2 daily doses, each of 20 mgm. per kg. body weight given intramuscularly. Tetanus antitoxin was administered as a single dose of 200 units per

TABLE VII

Comparative Effect of Terramycin and Antitoxin in Experimental Tetanus in Rabbits. Treatment Begun 24 Hours after Injection of Tetanus Spores

Rabbit No.	Type of Treatment	Results—Days after Infection	
		Development of first signs of tetanus	Death from tetanus
T 1	Terramycin only*	3	4
T 5		3‡	—
T 20		None§	—
T 17	Antitoxin only†	10	11
T 18		11	12
T 21		7	14
T 2	Terramycin* and antitoxin†	None	—‖
T 7		None	—
T 22		None	—
T 3	None—spores only	3	4
T 6		4	5
T 13		3	4

* Terramycin—20 mgm./kg. per dose for 2 daily doses.
† Tetanus antitoxin 200 units per kg. body wt. in 1 dose intramuscularly.
‡ Local tetanus only.
§ No symptoms of tetanus during observation period of 28 days.
‖ Animal died 10th day following cardiac puncture.

kg. body weight of a commercial preparation in horse serum. At 24 hours post-injection serum antitoxin levels varied from 2.0 to 4.0 units per ml.; on the seventh day all but one animal had less than 0.01 unit per ml. These rates of excretion are similar to those found in other studies in this laboratory (12).

The results of this experiment are shown in Table VII. The untreated animals developed rapidly progressive generalized tetanus in 3 to 4 days, and died within a few hours. One animal which received terramycin only developed generalized tetanus within 3 days and died about 78 hours after the injection of tetanus spores. Another animal which received terramycin only, developed a partial paralysis of the right hind leg on the third day, but the animal remained without other symptoms throughout the 28-day observation period. One animal remained symptomless throughout.

The three animals that received tetanus antitoxin remained symptomless for 7, 10, and 11 days, respectively. One animal developed typical signs of local tetanus in the right hind leg on the seventh day. Symptoms were slowly progressive; 2 days later there was evidence of extension and it was not until the eleventh day that generalized tetanus was noted; the animal succumbed on the fourteenth day. In the other two animals, generalized tetanus, while delayed until the tenth and eleventh day, respectively, was rapidly progressive.

By contrast, the animals that received both antitoxin and terramycin remained without signs of tetanus, although one animal died on the tenth day following cardiac puncture, apparently from hemopericardium.

DISCUSSION

The foregoing experiments provide some data bearing on the uses and limitations of antibiotics in the prophylaxis and treatment of tetanus. The combined *in vitro* and *in vivo* experiments indicate that terramycin is more effective against *Cl. tetani* than the other antibiotics tested, with penicillin exhibiting a probably useful range of effectiveness. The dosages which proved effective in the experimental disease are within the limits of the usual doses of these antibiotics in man.

It appears that these antibiotics are effective mainly against the vegetative forms of *Cl. tetani*, and not upon the spores as such, although in very large amounts penicillin did seem to have a lethal effect on the spore stage of the organism. (See Table II). If these findings are valid, then in the use of antibiotics following injury, the drug should be continued for a number of days in order that the vegetative forms may have an opportunity to develop. It should be recalled that germination and toxin production occur only under anaerobic or micro-aerophilic conditions, and such conditions following injury in man may not develop for 24 to 48 hours following trauma.

Once germinating tetanus bacilli have elaborated toxin, it is not to be expected that antibiotics could modify the action of the toxin itself, and this is probably the basis for the observed deaths when the administration of terramycin was delayed for 24 to 48 hours after the injection of spores. It has been suggested (5) that antibiotics may not reach the site of multiplication of *Cl. tetani*, since multiplication is believed to occur principally in damaged or necrotic tissue, but there is no convincing experimental or clinical evidence that this situation actually obtains. Proper surgical management of cases would tend to minimize this factor.

The results in Table VII indicate that a short course of terramycin can in fact eliminate tetanus infection. In animals that received tetanus antitoxin only, there was active infection present for 10 days or longer, since, upon excretion of most of the passively administered antitoxin, typical tetanus developed. By contrast, animals which received terramycin and survived the initial massive dose of spores did not relapse following cessation of treatment, indicating that viable tetanus organisms were no longer present. Similar experiments in mice confirm these results. (See Table VI).

With reference to the prophylactic value of tetanus antitoxin, the results reported here merely substantiate previous findings to the effect that the administration of antitoxin gives only temporary protection at best. In both rabbits

and mice which received tetanus antitoxin only, clinical tetanus developed when serum antitoxin fell to low levels.

It should be noted, too, that serum levels (2.0–4.0 units per ml.) observed in the rabbits to which relatively large doses of antitoxin were administered were about 10 times higher than that ordinarily reached in human beings given the usual prophylactic dose of 1500 units (0.2–0.4 units per ml.) (13). There are factors in the experimental situation which might make direct comparison with that in man invalid, particularly the large experimental inoculum of tetanus spores. Yet, the principles involved in the two situations seem to be essentially the same.

Finally, it should be noted that the technical procedure, incident to these studies, has been developed to the point that reasonably accurate quantitation of the infectious agent can readily be made, and that this can be employed to invoke a uniform type of disease which bears some resemblance to the naturally occurring disease in man. This has been accomplished largely through the production of large batches of spores which are then frozen at −70°C. in 15 per cent glycerin (14), thus preserving essentially unchanged the infectivity of the pool of organisms over long periods of time.

It is evident from the material presented in this paper and others from this clinic (13, 15) and from reviews of other work contained therein, that considerable new knowledge bearing on the prophylaxis and treatment of tetanus has accumulated since World War II. While most of this new knowledge has been obtained from laboratory experiments, nevertheless, it seems justifiable to apply some of the experimental results to clinical practice.

It is scarcely necessary to point out the great value of active immunization with tetanus toxoid. Most properly immunized persons show substantial serum antitoxin levels for 10 years or longer, and retain the capacity to respond promptly to a booster dose of toxoid (13, 15). Such individuals should not be given tetanus antitoxin. In the face of severe crushing injuries, however, additional protection would be afforded the patient by terramycin or penicillin given over a period of four days, which is the period required for a rise in antitoxin levels following a booster dose of toxoid.

For injured individuals who have not been previously actively immunized, terramycin and probably penicillin alone appear to offer greater degrees of protection than tetanus antitoxin alone. It is quite clear that antitoxin affords only temporary protection at best, while the antibiotics actually have the capacity to destroy tetanus organisms that might have contaminated a wound. It is recognized that practitioners will hesitate to discard a prophylactic procedure which since World War I has become traditional in traumatic surgery, yet the use of terramycin alone has a sound biologic foundation, and its use would seem to be justified on a trial basis. The administration of tetanus antitoxin is by no means an innocuous procedure and should be avoided whenever feasible. The

use of terramycin, likewise, may not be entirely without side effects, although on balance it is probably preferable to tetanus antitoxin in horse serum.

Finally, every person with a traumatic wound should be given a dose of tetanus toxoid, unless he has had one in the past 2–3 years. If the individual has had previous active immunization, a booster rise in serum antitoxin will be invoked; while if he has not had previous active immunization a dose of toxoid will begin the process.

The present study affords no direct observations on the effect of antibiotics on clinically manifest tetanus. While these drugs would of course be no substitute for tetanus antitoxin, since they would have no effect on toxin already formed, it would seem to be a rational procedure to give either terramycin or penicillin to such patients in order to eliminate multiplying tetanus organisms.

The foregoing studies, likewise, suggest new and practical approaches to certain problems in veterinary medicine.

SUMMARY

Study of the effects of antibiotics on *Clostridium tetani* both in culture and in the experimental infection in animals yielded the following results:

1. Using an inoculum of approximately 100 viable tetanus spores in thioglycollate broth cultures the mean values for the lowest inhibitory concentrations of various antibiotics in terms of micrograms per milliliter were as follows: achromycin 0.045; ilotycin 0.095; aureomycin 0.24; terramycin 0.16; chloromycetin 0.47; penicillin 0.6; streptomycin 7.5; magnamycin 10.0.

2. Evidence adduced indicated that the more effective of these antibiotics were bactericidal in their action on *Cl. tetani*. With the exception of penicillin, all antibiotics tested appeared to act only on the vegetative forms of tetanus organisms and not on the spores. In high concentrations, penicillin appeared to have a sporicidal effect.

3. In mice given approximately 1000 viable tetanus spores in 4.5 per cent calcium chloride intramuscularly, the daily dose of drug in milligrams per kilogram body weight of the animals required to protect half the infected animals was for terramycin 1.4; penicillin 8.1; aureomycin 10.6; ilotycin 11.6, achromycin 14.1. However confidence limits for all these except terramycin overlapped, indicating no significant difference in their relative effectiveness.

4. In experimental infections in hamsters when the antibiotic was given in 5 daily doses, individual doses of 20, 10 and 5 mgm./kg. of terramycin protected all animals. All animals were likewise protected by 80 mgm./kg. of penicillin. Penicillin in doses of 40 and 20 mgm./kg. each, and aureomycin in doses of 20 and 10 mgm./kg. afforded lesser degrees of protection.

5. In rabbits experimentally infected with approximately 40,000 tetanus spores in calcium chloride, complete protection was afforded by terramycin in both 2 and 4 daily doses of 20 mgm./kg. each, when the first dose was given

immediately after infection. Procaine penicillin in 1 dose and in 2 doses 48 hours apart of approximately 50–60 mgm./kg. delayed the onset of tetanus, but did not afford eventual protection.

6. Delay in the administration of either terramycin or penicillin until 24 hours or longer after infection with tetanus spores reduced the proportion of mice and rabbits protected, presumably because of early toxin production.

7. The administration of tetanus antitoxin to mice and rabbits experimentally infected afforded temporary protection, but most of the animals died after an interval of several days, presumably upon the excretion of most of the administered antitoxin.

8. In one experiment, rabbits, given both terramycin and tetanus antitoxin 24 hours after injection of tetanus spores did not develop tetanus, while those given antitoxin only eventually succumbed.

CONCLUSION

It is concluded that penicillin and the tetracycline group of antibiotics have a bactericidal action on *Clostridium tetani*, both *in vitro* and *in vivo*, and that this action is principally on the vegetative forms of the organism. Of the antibiotics tested terramycin and penicillin in doses comparable to those practicable in man, yielded the best results in protecting animals experimentally infected with tetanus spores.

Since tetanus antitoxin may give only temporary protection against the toxin produced by multiplying tetanus organisms, it would seem to be rational practice to give either terramycin or penicillin in appropriate doses, rather than tetanus antitoxin, in the attempted prophylaxis of tetanus, especially in those individuals not previously immunized. Administration of one of these antibiotics, in addition to tetanus antitoxin would seem to be a rational procedure in cases of clinical tetanus.

The authors wish to express their thanks to Miss Sara Prudovsky for assistance in part of these studies.

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SOME FACTORS INFLUENCING THE FORMATION OF L.E. CELLS

A Method for Enhancing L.E. Cell Production

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Received for publication October 4, 1955

The formation of L.E. cells is a phenomenon which occurs *in vitro* and which appears to be dependent upon the presence of three factors. One of these, the "plasma factor", is evidently a specific substance associated with the gamma globulin fraction of the plasma proteins of many patients with systemic lupus erythematosus (SLE). The other two factors, leukocytic nuclear material and actively phagocytic white blood cells, may be obtained from healthy as well as diseased individuals and from heterologous as well as homologous sources. Many investigators believe that alteration of cell nuclei to form hyalinized amorphous masses is a result of the specific action of the L.E. plasma factor, and that phagocytosis of these masses to produce L.E. cells is a secondary phenomenon. In positive L.E. cell preparations, extracellular masses of material resembling the intracellular inclusions are usually seen and at times are more numerous than L.E. cells. The "rosettes" so often encountered are probably composed of these same masses of altered nucleoprotein about to be ingested by surrounding leukocytes.

L.E. cells are rarely demonstrable *in vivo*, even though they may appear at a rapid rate in blood which has been removed from the body. A satisfactory explanation for this observation has not yet been provided. Several reports suggest that damaged leukocytes may be a particularly good source of the nuclear material required for L.E. cell formation. Thus, the use of outdated bank blood has been found to accelerate production of the cells (1). Kurnick and his coworkers (2) showed that washed leukocytes are more sensitive to L.E. cell transformation than unwashed cells. Gonyea and his associates (3) and Zimmer and Hargraves (4) demonstrated that the L.E. cell phenomenon is potentiated by coagulation of whole blood, an observation confirmed by a number of other investigators.

It seemed clear to us that the formation of L.E. cells requires the occurrence of contacts between cells and nuclear material. Furthermore, there was suggestive evidence that damaged cells provide a more ready source of nuclear material. Therefore a method was devised which agitates and at the same time traumatizes the blood cells. This technique consists of rotating heparinized

* This investigation was supported in part by a research grant of the Division of Research Grants and Fellowships, National Institutes of Health.

blood in a flask containing glass beads prior to the preparation of the buffy coat smears. The studies which are to be described are concerned with the experimental and clinical use of this method.

METHODS

*Rotary L.E. cell technique**

Five ml. of freshly drawn venous blood were placed in a dry test tube containing 1 mg. of sodium heparin. Within two hours 1.5 ml. of this blood were transferred to a 50 ml. Erlenmeyer flask containing 15 glass beads, 4 mm. in diameter. The flask was placed in a rotating device similar in design to that described by Shen and his associates (5) for determining the mechanical fragility of red cells. The flask was rotated at 40 r.p.m. for 30 minutes at a temperature of 37° C. After rotation 1.0 ml. of blood was transferred to a Wintrobe hematocrit tube and centrifuged at 1,000 r.p.m. for 10 minutes. The buffy coat layer was then pipetted off, smeared on cover slips, and stained with Wright's stain.

Clotted blood L.E. cell technique

A modification of the "two-hour blood-clot technic" described by Zimmer and Hargraves (4) was used. Five to 7 ml. of venous blood were allowed to clot at room temperature. At the earliest sign of clot retraction the clot was removed from the tube and cut in half. The lower portion was discarded and the upper half was fragmented with wooden applicators. The clot fragments were left at room temperature for a period of one hour and were transferred to a Wintrobe hematocrit tube. The remainder of the technique was similar to that used in the rotary L.E. cell method.

Heparinized blood L.E. cell technique

The method used was the same as that described by Suksta and Conley (6).

Quantification of the L.E. cell phenomenon

Each of a pair of cover slip smears was scanned systematically with the high power objective for a period of 15 minutes. If no L.E. cells, rosettes or extracellular material were seen, the preparation was reported as negative. If L.E. cells were seen, then 500 consecutive polymorphonuclear granulocytes were counted on each cover slip. The degree of L.E. cell formation was expressed as the number of L.E. cells per 1,000 polymorphonuclear granulocytes. Polymorphonuclear granulocytes grouped as rosettes were included in the 1,000 cells counted. The amount of rosette or extracellular material formation was graded as rare, 1+, 2+, 3+, and 4+.

Plasma fibrinogen

The method of Ratnoff and Menzie (7) was used for determination of plasma fibrinogen. Duplicate determinations were performed on the same sample of heparinized plasma, and

* Results comparable to those obtained with the mechanical fragility device were achieved with several commercially available instruments. One device suggested by Dr. George Brecher is the Bryan-Garrey Blood Cell Pipette Rotor (A. S. Aloe Company). Test tubes (½″ x 4″) containing 3 ml. of heparinized blood and 5 glass beads are rotated on this machine for 30 minutes. Another agitating device which can be used is the platform style Kahn shaker. Petri dishes containing 3 ml. of heparinized blood and 15 beads are agitated for 30 minutes.

the value reported was the average of these results. In several experiments simultaneous determinations on oxalated and heparinized plasma specimens yielded similar results.

RESULTS

1. *Effect of rotation on normal blood and blood from patients with diseases other than systemic lupus erythematosus*

Heparinized venous blood from 20 normal adults was rotated with glass beads by the technique which has been described. In the smears prepared from the buffy coats cellular yields were excellent and morphologic features were surprisingly well preserved (Fig. 1a). These smears were hardly distinguishable from those prepared from blood which had not been rotated. Leukocytes were uniformly distributed but large clumps of platelets were often present. In the few experiments in which sequestrene was used as the anticoagulant, there was no evidence of platelet clumping after rotation. The degree of nucleophagocy-

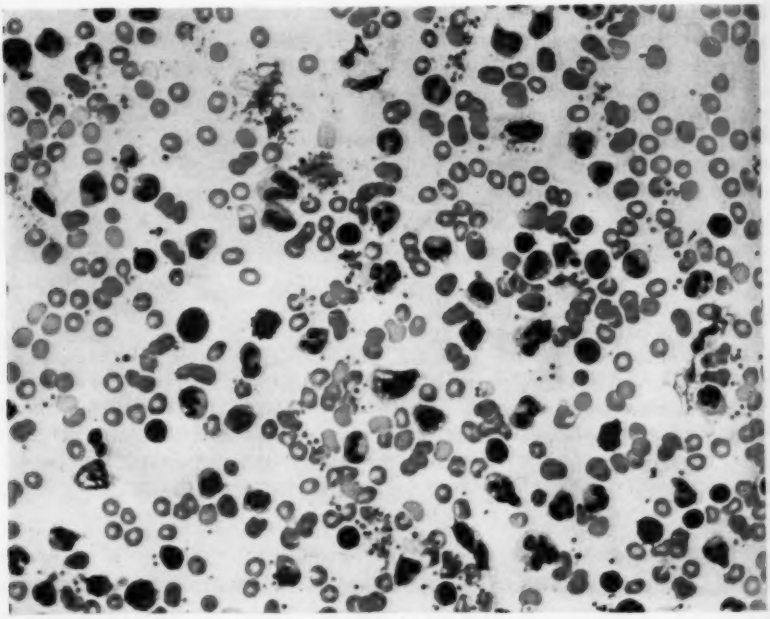

FIG. 1a

FIG. 1. Photomicrographs of smears of buffy coat of heparinized blood stained with Wright's stain. a. Rotated blood from a normal adult. (×400) b. Blood incubated but not rotated from a patient with SLE. There are large clumps of platelets but no extracellular material and only rare L.E. cells. (×200) c. Rotated blood from the same patient as in *b*. Many L.E. cells are apparent. (×200) d. Blood incubated but not rotated from a patient producing only extracellular material. (×400) e. Rotated blood from the same patient as in *d*. Large amounts of extracellular material are present. (×400)

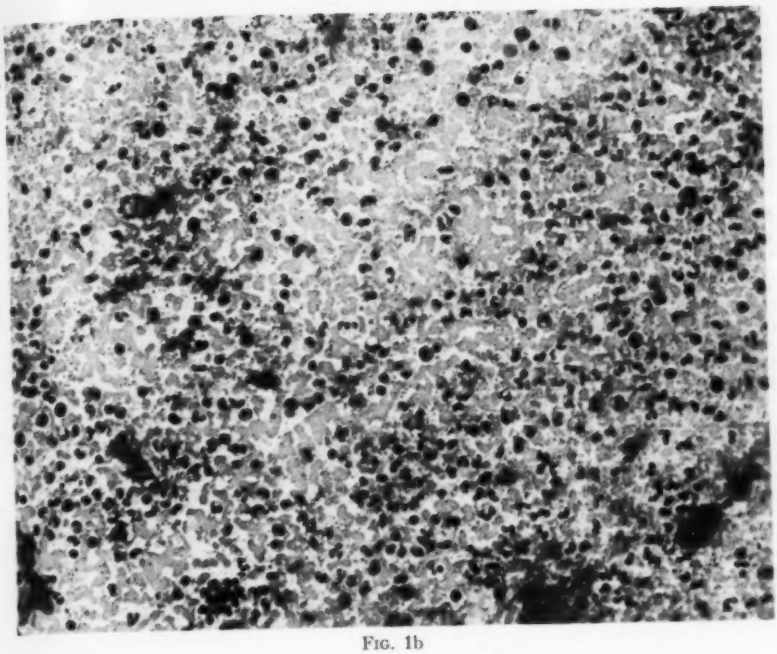

FIG. 1b

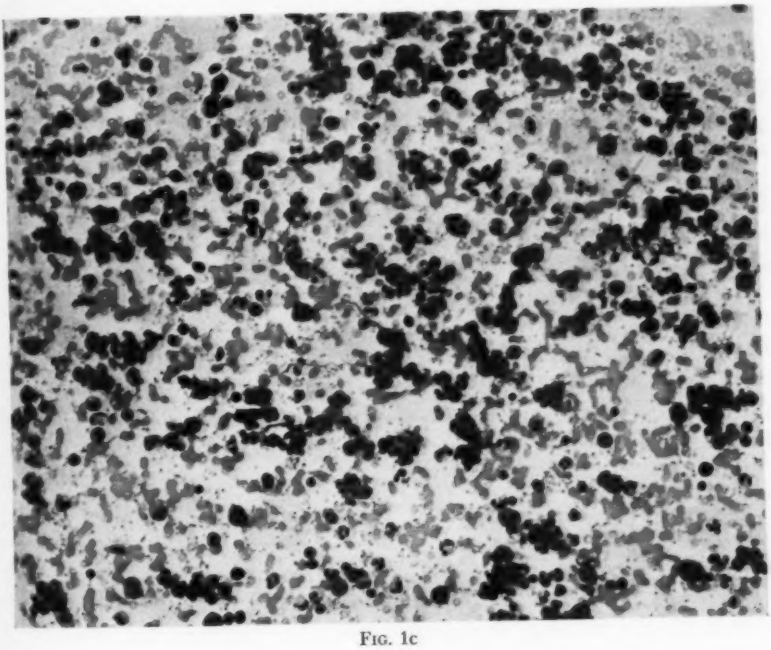

FIG. 1c

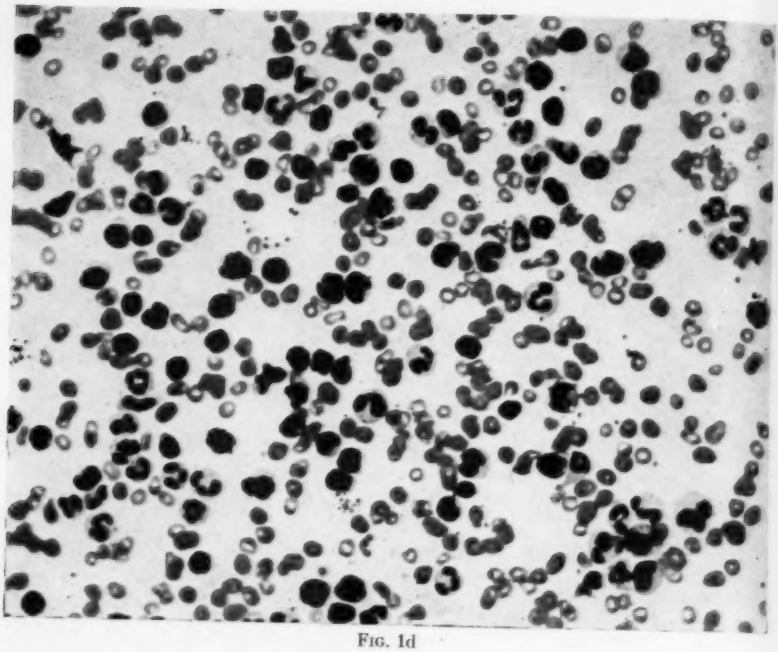

FIG. 1d

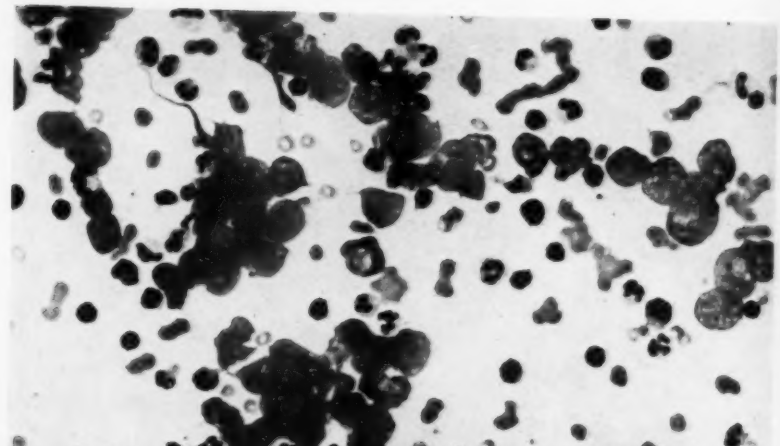

FIG. 1e

tosis and of erythrophagocytosis was comparable to that observed in the control specimens which had been incubated but not rotated. Only a very rare nucleophagocyte was seen and the number of erythrophages was far less than one per 1,000 phagocytic leukocytes. In some of the smears a few strands or meshes of fine red-purple material were observed. This substance, possibly fibrin, did not resemble the amorphous masses of extracellular material seen in positive L.E. cell preparations.

The rotation technique was applied to the blood of 132 patients with diseases other than systemic lupus erythematosus. Many of these patients had disorders of the "collagen-vascular" group including rheumatoid arthritis, scleroderma, periarteritis nodosa and acute rheumatic fever. Others had essential hypertension, erythema nodosum, erythema multiforme, penicillin hypersensitivity, chronic urinary tract infections, "idiopathic" acquired hemolytic anemia, meningovascular syphilis, Raynaud's syndrome and "idiopathic" thrombocytopenic purpura. In each instance the smears failed to show structures resembling L.E. cells or extracellular material.

2. *Effect of rotation on blood from patients with systemic lupus erythematosus*

Blood was obtained from 16 patients who had been demonstrated to produce L.E. cells by standard techniques. All of these patients were thought to have SLE on clinical grounds. A portion of the heparinized specimen was rotated; another portion incubated but not rotated served as the control. The results of this study are shown in Table I. In most instances there was a striking increase in the number of L.E. cells and amount of extracellular material after rotation (Fig. 1b, 1c). When L.E. cells were found only after prolonged search in incubated preparations, they were often numerous after rotation. In three cases L.E. cells were demonstrable by the rotation technique when they were not found in the non-rotated incubated preparations. On one occasion no L.E. cells were seen in the rotated preparation but in this instance there was a large amount of extracellular material typical of positive preparations.

Blood from 11 of these patients was subjected to study simultaneously by the rotation and clotted blood techniques. Comparison of the results showed that in most cases L.E. cells were more numerous in the rotated specimens. However, in three cases the clotted blood technique produced more L.E. cells. For unknown reasons variations in the degree of positivity were observed when the same technique was repeated. Thus, on two occasions L.E. cells were seen in the clotted preparation but not in smears from rotated blood, although L.E. cells were demonstrated by the rotation technique in blood obtained subsequently from these same patients.

During the period of this study ten additional patients were encountered who had typical clinical and laboratory evidences of SLE except for repeatedly negative L.E. cell tests. In four of these cases, L.E. cells had been demon-

TABLE I

Effect of Rotation on Heparinized Blood from Patients with SLE. Comparison of This Method with Heparinized and Clotted Blood Techniques

Patient	Date	Methods						Comment
		Heparinized L.E. technique		Rotary L.E. technique		Clotted blood L.E. technique		
		L.E. cells 1000 PMN	Ecm.*	L.E. cells 1000 PMN	Ecm.*	L.E. cells 1000 PMN	Ecm.*	
L. A.	12–23–54	3	Rare	114	2+			Oral cortisone, 300 mg. a day started on 12–29–54. Decreased gradually to 100 mg on 1–24–55, and discontinued 2–18–55
	2–9–55	<1	Rare	<1	3+			
	2–12–55	<1	Rare	2	1+			
	2–25–55	<1	0	6	1+			
	3–8–55	2	Rare	62	2+			
	3–17–55	3	Rare	59	3+			
B. R.	2–5–55	<1	Rare	52	2+			No steroid therapy
A. W.	1–28–55	0	Rare	14	4+			No steroid therapy
J. H.	2–7–55	<1	Rare	10	3+			No steroid therapy
A. B.	5–5–55	1	0	26	2+	8	2+	On cortisone, 75 mg. a day
E. C.	2–4–55	<1	Rare	0	4+			On cortisone, 75 mg. a day
	5–16–55	1	Rare	100	1+	37	1+	
M. B.	4–21–55	3	0	89	2+	104	1+	On ACTH Gel, 20 u a day
G. N.	4–21–55	0	0	0	Rare	<1	Rare	
	5–26–55	0	0	7†	2+	<1	Rare	No steroid therapy
W. R.	3–21–55	<1	Rare	202	1+	79	2+	On hydrocortisone, 60 mg. a day, from 2/14/54 to 3/29/55. Prednisone, 50 mg. a day started on 3/29/55, decreased to 25 mg. a day on 4/10/55, and discontinued on 4/15/55
	4–1–55	1	Rare	103	2+			
	4–15–55	<1	Rare	175	Rare			
	4–28–55	1	0	109	Rare	64	1+	
	5–9–55	1	0	104	Rare			
B. T.	4–11–55	<1	0	263	1+	91	2+	On cortisone, 75 mg. a day
G. S.	4–4–55	<1	Rare	352	3+	256	1+	No steroid therapy
F. K.	4–2–55	0	Rare	0	2+	13	1+	No steroid therapy
	4–6–55	0	0	<1	0			

TABLE I—*Continued*

Patient	Date	Methods						Comment
		Heparinized L.E. technique		Rotary L.E. technique		Clotted blood L.E. technique		
		L.E. cells 1000 PMN	Ecm.*	L.E. cells 1000 PMN	Ecm.*	L.E. cells 1000 PMN	Ecm.*	
S. M.	5–10–55	<1	Rare	8†	1+			No steroid therapy
V. S.	5–10–55	<1	Rare	31	1+	11	Rare	No steroid therapy
G. B.	5–13–55	<1	Rare	4	2+	237	2+	No steroid therapy
M. H.	6–6–55	<1	Rare	1+	2+	3	1+	No steroid therapy

* Extracellular material.

† Blood rotated in Bryan-Garrey blood cell pipette rotor.

strated at an earlier stage of the disease. Repeated examination of the blood of these patients by the rotation and clotted blood techniques gave uniformly negative results.

In two cases the rotary method was used to study the effect of steroid therapy on the L.E. cell phenomenon (Table I, Cases L. W. and W. R.). One of these patients showed a striking decrease in number of L.E. cells formed, with reappearance of the cells as the dose of cortisone was reduced and eventually discontinued. In the other case increasing the amount of steroid had no measurable effect on L.E. cell formation. Both of these patients had a satisfactory clinical response to steroid therapy. Several other patients produced L.E. cells while receiving hormonal therapy.

3. *Factors influencing the number of L.E. cells produced by rotation*

Experiments were performed in an effort to analyze the mechanism by which rotation potentiates L.E. cell production. In this study observations were made on the effect of varying rate and duration of rotation, number of glass beads, amount of blood and character of the surface.

Relation of number of beads and rate of rotation to number of L.E. cells formed: Blood obtained from a patient with SLE was heparinized and immediately divided into aliquots. One portion was incubated but not rotated. The remainder was divided into 14 flasks, so that each contained 1.5 ml. All flasks were rotated for 30 minutes. The number of 4 mm. glass beads and the rate of rotation for each flask are shown in Fig. 2. When heparinized blood was rotated without glass beads, the number of L.E. cells formed was no greater than in the non-rotated controls. When glass beads were present, there was a pronounced increase in L.E. cell production. The number of cells formed was directly related to the number of beads in the rotating flask. When more than 15 beads were

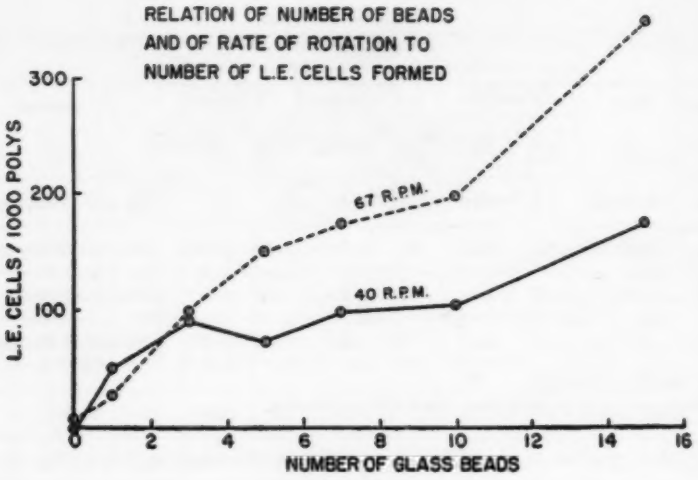

FIG. 2

added, cellular distortion and fragmentation were so prominent that it was impossible to quantify L.E. cell formation. Increasing the rate of rotation from 40 r.p.m. to 67 r.p.m. increased the number of L.E. cells when more than three beads were present.

Relation of duration of rotation to number of L.E. cells formed: The general plan of this experiment was similar to that described above. One and one-half ml. portions of heparinized blood from a patient with SLE were rotated at 40 r.p.m. in three series of four flasks. The first series contained no beads, the second contained one 4 mm. glass bead, and the third three beads in each flask. The duration of rotation of each of the four flasks in each series is shown in Fig. 3. As in the previous experiment there was no enhancement of L.E. cell production when blood was rotated without glass beads, even though rotation was continued for two hours. When beads were present, the number of L.E. cells increased with the time of rotation. When flasks containing beads were rotated for more than two hours, there was so much destruction of leukocytes that accurate counts could not be performed.

Relation of amount of blood rotated to number of L.E. cells formed: In this experiment, which was patterned after those described, the amount of blood in the rotating flask was varied. Four series of four flasks were rotated at 40 r.p.m. for 30 minutes. The volume of blood and number of beads in each flask are shown in Fig. 4. When blood was rotated without beads, there was again no increase in L.E. cell formation, even when use of a small amount of blood permitted a relatively greater blood-glass interface. When beads were present, there was an inverse relationship between the volume of blood employed and the number of L.E. cells formed.

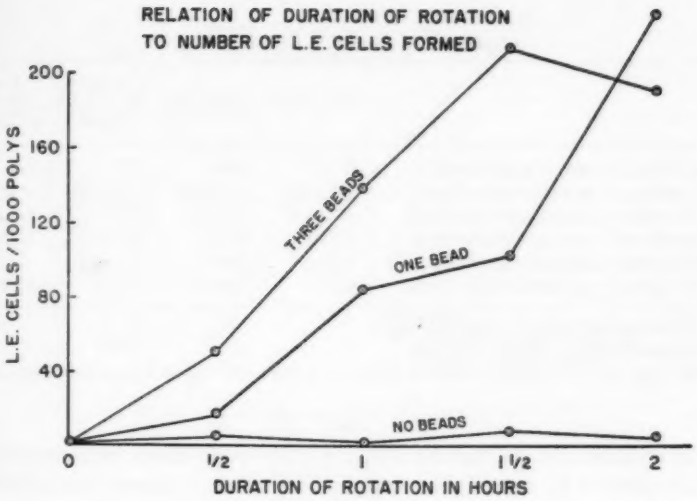

FIG. 3

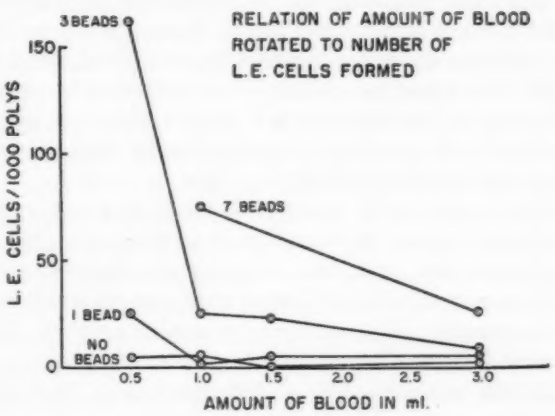

FIG. 4

Role of glass surfaces in the potentiation of L.E. cell formation by rotation: These experiments were designed to elucidate the role of glass surface *per se* in L.E. cell formation.

To determine if glass contact affects specifically the plasma factor or the leukocytes, these were rotated separately. Thirty ml. of blood from a patient with SLE were added to 6 mg. of dry sodium heparin and centrifuged in an International Type SBV centrifuge at 3,000 r.p.m. for 15 minutes. The supernatant plasma was removed and the buffy coat drawn off with a capillary pipette. Half of the buffy coat was washed three times with 0.85% sodium

TABLE II

Portion of L.E. System Affected by Rotation

	Non-rotated	Plasma and Buffy Rotated	Buffy Rotated and Added to Non-rotated Plasma	Plasma Rotated and Added to Non-rotated Buffy
Control plasma + control washed buffy.....	0*/0†	0/0	0/0	0/0
Control plasma + patient's washed buffy....	<1‡/rare	<1‡/rare	<1‡/rare	<1‡/rare
Patient's plasma + control unwashed buffy...	<1/rare	111/4+	26/2+	9/rare
Patient's plasma + control washed buffy.....	12/1+	92/4+	44/2+	12/2+
Patient's plasma + patient's unwashed buffy.	1/rare	104/3+	—	—
Patient's plasma + patient's washed buffy...	42/2+	158/4+	51/2+	47/2+

* Indicates number of L.E. cells/1000 polys.

† Indicates degree of rosette formation.

‡ This degree of L.E. activity was seen in patient's buffy coat before being used in this experiment.

chloride solution and the washed and unwashed buffy coats were resuspended in saline solution to the original volume. Heparinized plasma and unwashed and washed buffy coats were obtained in a similar manner from a normal adult. Various mixtures of normal and patient's plasma and buffy coats were made before and after rotation as shown in Table II. Maximum L.E. activity occurred when patient's plasma and either normal or patient's buffy coat were rotated together. There was a moderate increase in L.E. activity when buffy coats were rotated separately and then added to L.E. plasma. Except in one instance the addition of rotated L.E. plasma to non-rotated buffy coats produced no more L.E. cells than seen in the non-rotated controls.

To determine if exposure of heparinized blood to rotating glass surfaces caused defibrination, plasma fibrinogen levels were measured before and after rotation. As shown in Fig. 5 there was no significant change in plasma fibrinogen after rotating normal blood and blood from patients producing L.E. cells or extracellular material. None of the seven patients making L.E. cells had more than 2 per 1,000 granulocytes before rotation. After rotation the average number of L.E. cells formed was 65 per 1,000 granulocytes. Both of the patients making extracellular material had only a rare piece before rotation and 4+ afterwards.

Other studies were designed to demonstrate whether an earlier phase of the coagulation process is initiated by exposure of heparinized L.E. blood to rotating glass surfaces. L.E. cell plasma from which prothrombin was removed by adsorption on barium sulfate produced L.E. cells when rotated with washed buffy coats. It has been shown that barium sulfate-adsorbed plasma is also deficient in plasma thromboplastic component (8).

To study the effect of siliconized glass surfaces on number of L.E. cells formed by rotation, the following experiment was done. Heparinized venous

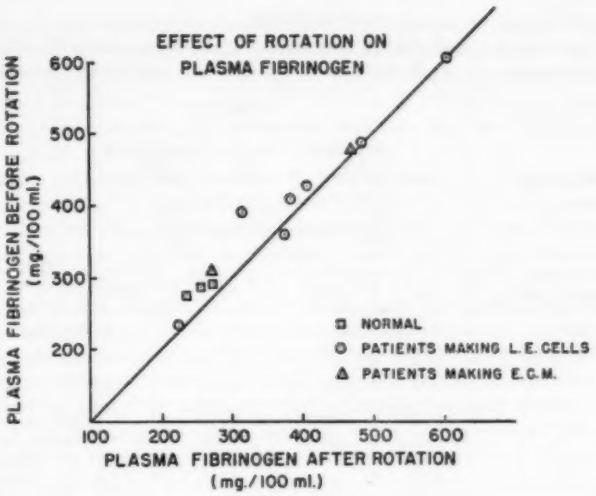

FIG. 5

blood was obtained from three patients whose plasma contained the L.E. cell promoting factor. Syringes were treated with silicone and blood was placed in siliconized glass tubes. Two flasks were rotated for each specimen of blood; in one beads and flask were treated with silicone, and in the other beads and flask were not siliconized before rotation. When flask and beads were siliconized there was a moderate to marked reduction in L.E. cell formation. Without silicone, 87, 263, and 202 L.E. cells per 1,000 polymorphonuclear granulocytes were produced. After siliconization, 1, 51 and 36 L.E. cells per 1,000 polymorphonuclear granulocytes respectively were observed. White blood cells on smears from the siliconized preparations were better preserved than those from the non-siliconized flasks, although some of the white cells had large vacuoles. These vacuoles were larger than L.E. cell inclusions, and only a rare piece of extracellular material was present on these smears. The flow characteristics of the blood during rotation in the siliconized and non-siliconized systems were quite different. Blood in the siliconized flasks cohered into one large globule, while in the others the blood formed a thin film between the beads and flask wall.

4. *Effect of rotation on blood from patients forming only extracellular material*

Ten patients were encountered who formed only extracellular material with the heparinized, rotary, and clotted blood techniques on repeated examinations (Table III). Rosettes and L.E. cells were never seen in these preparations. The clinical features and laboratory findings in many of these cases were compatible with the diagnosis of SLE. Two of the patients had L.E. cells demon-

TABLE III

Effect of Rotation on Heparinized Blood from Patients Making Extracellular Material (ECM) and Comparison of This Method with Heparinized and Clotted Blood Techniques

Patient	Diagnosis	Date	Method						Comment
			Heparinized L.E. technique		Rotary L.E. technique		Clotted blood L.E. technique		
			L.E. cells/ 1000 pmn.	Ecm.	L.E. cells/ 1000 pmn.	Ecm.	L.E. cells/ 1000 pmn.	Ecm.	
C. T.	Recurrent pericarditis	5-19-55	0	Rare	0	4+	0	Rare	
J. H.	SLE	2-24-55	0	1+	0	3+			On cortisone, 75 mg. a day
		3-31-55	0	Rare	0	4+			
		4-14-55	0	1+	0	3+			
		5- 5-55	0	Rare	0	3+	0	1+	
B. M.	SLE	3-10-55	0	Rare	0	3+			Cortisone, 300 mg. a day, started on 3-7-55, decreased to 200 mg. a day on 3-15-55, and tapered to 75 mg. a day on 4-4-55
		3-15-55	0	Rare	0	4+			
		3-22-55	0	Rare	0	4+	0	1+	
		3-29-55	0	1+	0	4+			
		4- 5-55	0	Rare	0	4+			
F. P.	SLE	3-14-55	0	Rare	0	3+	0	1+	No steroid therapy
R. H.	SLE	4- 4-55	0	Rare	0	3+	0	1+	No steroid therapy
L. F.	Arthritis, thrombophlebitis and splenomegaly	6- 8-55	0	1+	0	3+	0	1+	No steroid therapy
J. K.	Hypertension treated with Hydralazine (apresoline)	6- 7-55	0	Rare	0	3+	0	1+	No steroid therapy
M. M.	SLE	2-15-55	0	0	0	4+			No steroid therapy
E. R.	Chronic pain in legs	5-20-55	0	Rare	0	3+	0	1+	Prednisone discontinued three weeks before this observation
A. H.	Acquired hemolytic anemia of 2 years' duration	8- 2-55	0	Rare	0	3+	0	1+	Prednisone, 60 mg. a day, started on 8-4-55, decreased gradually to 40 mg. a day on 8-23-55
		8-11-55	0	Rare	0	3+			
		8-16-55	0	Rare	0	3+			
		8-19-55	0	Rare	0	3+			
		8-23-55	0	Rare	0	3+			

strated prior to the period of this study. In both of these, extracellular material had been repeatedly observed before L.E. cells were discovered. The case summaries of two patients whose clinical pictures were not typical of SLE are recorded:

Case I. L. F. (706765), a 42 year old white coal miner, was referred for examination because of the discovery of an enlarged spleen. Eighteen years before admission the patient developed thrombophlebitis of both legs while working in the mines. Several months later chronic leg ulcers appeared. Eight and six years before admission the patient had episodes of chest pain associated with chills, fever, cough, and dyspnea. For several years he had noted an irregular heart beat with attacks of tachycardia, facial flush, and "blacking out spells" lasting several seconds. Because of these attacks the patient was started on digitoxin.

For many years he had had transient arthralgia with erythema, swelling and heat involving the interphalangeal joints of the hands. Five weeks before admission he developed a sharp pain in the left upper quadrant of the abdomen. The pain was constant and aggravated by coughing or deep breathing. When seen by his physician he was told that he had a large and tender spleen. During the next few weeks the abdominal pain and tenderness gradually diminished, and the size of the spleen decreased.

On admission the temperature was 98.6° F, pulse 88, respirations 18 and blood pressure 130/75. The patient was well developed and well nourished and did not appear ill. A faint, blotchy erythematous eruption was present over the malar eminences, the shoulders, and anterior chest. From the ankles to the hips were areas of darkly pigmented and thickened skin. A few medium rales were heard over both lung bases. A soft systolic murmur was noted to the left of the sternum. Dilated, tortuous veins were present over the lower abdominal quadrants. A firm, smooth, non-tender liver was palpated 4 cm. below the costal margin. The spleen extended to the iliac crest and was smooth, very firm, and non-tender.

There was no anemia. The white cell count was 8,300 with 33% segmented neutrophils, 53% lymphocytes, and 15% monocytes. Sedimentation rate was 36 mm. in one hour (Wintrobe). S.T.S. was negative. Total serum protein was 7.3 grams per 100 ml., albumin 4.1 and globulin 3.2. Electrophoretic pattern of the serum was normal. Total bilirubin was less than 0.8 mg. per 100 ml. Thymol turbidity was 0.8 units and cephalin cholesterol flocculation was negative. Urinalysis was normal. Electrocardiogram revealed low T waves in V_4. Chest X-ray and gastrointestinal series were normal.

Case II. E. R. (704735), a 39 year old white waitress, was admitted for investigation of attacks of weakness and severe pains in both legs. Ten years previously the patient had several episodes of trench mouth. At this time aching and weakness of the legs were noted. The pain continued and four years later small, red, raised, itching lesions appeared over the anterior tibial areas. These lesions disappeared spontaneously in three months. Two years later she had generalized weakness, was transiently unable to stand without assistance, and aching in the legs became so severe that bed rest was necessary. At this time she was treated with adrenal extract, and she continued to take adrenal hormones until 3 weeks prior to admission.

Physical examination revealed a temperature of 99° F, pulse 76, respirations 16, and a blood pressure of 115/60. The patient was a well developed and well nourished woman not appearing ill. The skin was not remarkable. Heart and lungs were normal to percussion and auscultation. The liver and spleen were not palpated. There was no tenderness of the lower extremities.

Examination of the blood revealed a normal hematocrit value, white cell count and differential count. Sedimentation rate was 12 mm. Total serum protein was 6.5 gm. per 100 ml. with an albumin of 4.4 gm. Electrophoretic pattern of the serum was normal. Chest X-ray was normal. Urinalysis was negative.

When the rotary technique was applied to blood from this group of patients there was a tremendous increase in the amount of extracellular material (Fig. 1d, 1e). This material could not be differentiated from the free extracellular masses seen in preparations containing L.E. cells. Because of the large amount of material produced by rotation, many discrete globules of material coalesced to form large masses twenty to forty times the size of leukocytes (Fig. 1e). White blood cells were well preserved even in areas where the concentration of extracellular material was greatest.

To determine the origin of this material a number of experiments were per-

formed. No extracellular material was formed when plasma or washed buffy coats of these patients were rotated separately. Extracellular material was produced, however, when patient's plasma and washed or unwashed normal buffy coats were rotated together. No L.E. cells were seen in these preparations. The addition of plasma from a patient with SLE to washed and unwashed buffy coats from these patients produced L.E. cells, indicating that white cells from these patients were susceptible to L.E. cell transformation. When unstained smears were incubated with desoxyribonuclease the extracellular material disappeared*. Two patients were observed on treatment with steroids. Within two weeks the character of the extracellular material altered, changing from a homogeneous to a fine, lacy appearance.

DISCUSSION

The absence of L.E. cells from the circulating blood and their rapid appearance following removal of blood from the patient suggests (1) that L.E. cells, if formed *in vivo*, must be immediately removed from the circulation or (2) that L.E. cell production requires some special circumstances which are present *in vitro* but not *in vivo*. There is little to indicate that the first hypothesis is correct. Structures resembling L.E. cells have rarely been seen in biopsy or autopsy specimens from patients whose blood produced large numbers of L.E. cells *in vitro*. It is necessary to assume, therefore, that L.E. cells produced in the circulating blood must be immediately destroyed. In view of the tremendous number of granulocytic cells involved in L.E. cell formation in the blood removed from some patients with SLE, it seems unlikely that the leukocyte turnover rate could be so rapid.

Two theories have been proposed to account for the L.E. cell phenomenon. According to one the L.E. plasma factor is an "autoimmune" substance which simulates an anti-leukocyte antibody. In the presence of the L.E. factor white blood cells are sensitized and phagocytized by other leukocytes. According to this hypothesis, phagocytosis is the primary process, and the formation of the intracellular inclusion is the result. Evidence to support this belief is largely limited to a study of the appearance of white cell preparations following incubation with anti-leukocyte serum (9, 10). Evidence of leukophagocytosis is abundant in such preparations, and it has been claimed that structures resembling L.E. cells are present. However, there is considerable doubt that the inclusion bodies in these preparations are similar to those of L.E. cells and it seems more likely that they are simply ingested cell nuclei. A more generally accepted theory is that the L.E. plasma factor induces a specific chemical change in the nuclei of leukocytes, possibly depolymerization of desoxyribosenucleic acid

* Experiments with desoxyribonuclease were performed in collaboration with Dr. A. R. Rich and Dr. F. G. Germuth, Jr. and will be described fully in a subsequent report.

(11). The altered nuclei are subsequently ingested by other leukocytes to form L.E. cells. This concept explains the distinctly altered appearance and staining characteristics of the inclusion bodies, and also accounts for the presence of extracellular material and rosettes. It is not known whether the nuclear alteration may occur in living cells, but certain evidence suggests that damaged cells are more susceptible (1, 2, 3, 4). The need for dead leukocytes for L.E. cell production is emphasized by the recent report of Snapper and Nathan (12). If it is assumed that the L.E. plasma factor is unable to attack intact living cells, then the absence of L.E. cells from the circulation can be explained. Presumably leukocytes are removed at the end of their life span in such a way as to preclude their participation in the formation of L.E. cells. In the test tube, on the other hand, dying leukocytes are readily available as the source of nuclear material for the L.E. transformation.

Evidence supporting the latter hypothesis is provided by the present experiments. When blood was agitated in such a way as to avoid trauma to leukocytes, L.E. cell production was not enhanced, although contacts between cells must have been greatly increased. On the other hand, addition of glass beads to the rotating preparation had a spectacular effect, which seems best explained on the basis of injury to the white cells. No evidence was obtained to indicate that glass surfaces had a significant effect on the plasma, and specifically the clotting process appeared not to be involved. The tendency of silicone surfaces to reduce L.E. cell production in the rotating preparation is most likely attributable to the altered flow characteristics, with resultant decrease in trauma to cells.

Of particular interest in the present study is the "extracellular material" so regularly found in positive L.E. cell preparations. This amorphous, hyaline, globular substance, identical in staining characteristics with the L.E. cell inclusion, is lysed by desoxyribonuclease. It seems likely that the formation of this material, under the influence of the L.E. plasma factor, is the primary stage in L.E. cell formation. In all probability the material is derived from the nuclei of injured or dead leukocytes. L.E. cells are formed when this material is ingested by normal phagocytic leukocytes. It is noteworthy that the ratio between the amount of extracellular material and the number of L.E. cells differs in different patients. Blood from some patients makes many L.E. cells with little extracellular material. In other cases the extracellular material may be abundant although L.E. cells are rare or absent. Some patients have been encountered whose blood regularly produces extracellular material, but fails to form L.E. cells. All of these patients have not been proved to have SLE although most have clinical manifestations compatible with that disease. The studies which have been described show that the formation of extracellular material is the result of an abnormality of the patient's plasma. The extent to which L.E. cells are formed appears to be a property of the extracellular material. Evi-

dently this substance incites phagocytosis to varying degrees, presumably depending upon variations in its composition. Failure to form L.E. cells cannot be attributed to defective phagocytic activity of the patient's leukocytes, since L.E. cells are produced in abundance upon addition of the plasma of a patient whose blood forms L.E. cells. Valuable information might be provided by a chemical study of the extracellular material from various patients. It is possible that specific identification of this material might have more pathognomonic significance than the demonstration of L.E. cells. It also seems likely that the extracellular material from patients forming L.E. cells might be demonstrably different from that obtained from patients whose blood fails to form these cells. The rotary method makes possible the production of extracellular material in relatively large quantities, and should facilitate a histochemical approach to this problem.

The use of the rotary technique has facilitated the examination of blood for L.E. cells and is now in routine use at the Johns Hopkins Hospital. In a few instances, L.E. cells have been demonstrated by this method when they had not previously been found. However, it is important to emphasize that some patients with SLE fail to produce L.E. cells or extracellular material with any currently available technique. The significance of extracellular material in the absence of L.E. cells remains to be determined. The absence of extracellular material from preparations of blood from normal individuals and from patients with many diseases other than SLE makes it seem likely that demonstration of this material has some diagnostic import, but whether it is pathognomonic of SLE is not established.

SUMMARY

The production of L.E. cells in heparinized blood of patients with SLE is greatly increased when the blood is rotated with glass beads. Experimental studies suggest that this effect is attributable to trauma to the leukocytes which provide nuclear material for transformation. It seems likely that viable leukocytes are not subject to the influence of the L.E. plasma factor.

The rotary technique has been valuable as a clinical method for the detection of L.E. cells. Of particular interest is the large amount of "extracellular material" which may be produced by this technique, at times in the absence of L.E. cells. The use of this method should facilitate histochemical study of this altered nucleoprotein.

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THE PATHOLOGY AND PATHOGENESIS OF EXPERIMENTAL ANAPHYLACTIC GLOMERULONEPHRITIS IN RELATION TO HUMAN ACUTE GLOMERULONEPHRITIS

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Received for publication October 10, 1955

It has long been suspected that hypersensitivity may be an important determining mechanism in the pathogenesis of many of the instances of human glomerulonephritis. Particularly suggestive in this regard have always been post-scarlatinal nephritis, and the nephritis that is associated with tonsillitis, both of which, as is well known, do not appear during the height of the infection when the streptococci and their products are present in greatest abundance, but only after a lapse of time sufficient to permit the development of antibody and of hypersensitivity. In the early part of this century, Schick (1) and others compared this delay in the appearance of scarlatinal nephritis to the situation in serum sickness, in which the presence of the foreign antigen in the circulation provokes no symptoms until sensitizing antibody has been produced, at which time the symptoms and lesions of serum sickness make their appearance. The analogy with serum sickness seemed particularly apt when it became recognized that in serum sickness there commonly occur, though usually in mild form, disturbances of renal function of the type encountered in glomerulonephritis (2).

That glomerulonephritis need not result from bacterial products possessing primary toxicity, but may result from anaphylactic hypersensitivity to nontoxic substances, has been indicated by clinical observations apart from those related to actual serum sickness. Longcope and Rackemann (4), for example, described the case of a man who became hypersensitive to beef protein, and developed giant urticaria when he ate beef. The urticarial attacks were accompanied by albumin and casts in the urine, an increase in blood urea, profound depression of urea excretion, decrease in phthalein excretion, and retention of chlorides and water, with return to normal after the attacks. Ehrström (5) has reported two similar cases in patients hypersensitive to other food allergens, in one of whom the attacks were accompanied by hematuria, urinary casts, hypertension and edema.

In 1913, Longcope (6) reported the presence of renal lesions in animals that had been subjected to repeated injections of small amounts of foreign protein. He described, in some of the animals, swelling and proliferation of the glomerular epithelium, with occasional mitoses. While it is probable, from the results of more recent studies, that lesions of glomerulonephritis occurred in

some of the animals in those early experiments, all of the numerous photomicrographs show only lesions which are now recognizable as the focal, interstitial inflammatory lesions that occur frequently in the kidneys of untreated stock animals, particularly rabbits, and which, though they may damage glomeruli and tubules that happen to be situated within the inflammatory areas, are altogether different from true glomerulonephritis. Indeed, Longcope (6) stated, regarding the glomerular lesions, that "the most marked alterations occurred in those glomeruli that were situated in the cellular areas".

During the succeeding thirty years there were occasional other reports of the production of glomerulonephritis by repeated injections of foreign protein. Doubt was, however, always cast upon those reports, because the illustrations did not provide convincing evidence that glomerular lesions characteristic of those of human glomerulonephritis had been produced. In 1934, for example, Masugi and Sato (7) described glomerular alterations in several rabbits that had been given repeated intravenous injections of foreign protein after the removal of one kidney. The photomicrographs, which are quite clear, show alterations very different from those characteristic of ordinary human glomerulonephritis. They also injected 2–5 cc. of foreign protein directly into the renal artery of rabbits previously sensitized to the protein. This procedure, as might be expected, produced marked damage to the renal arterioles and glomerular capillaries, with focal infarction, for the experiment represented, in effect, the production of an Arthus phenomenon in the kidney, i.e., the severe vascular damage and thrombosis that results from bringing concentrated antigen directly into contact with the blood vessels of any tissue in the sensitized body. In other experiments Masugi (8) found that glomerular damage, strikingly similar to that in human glomerulonephritis, can be produced by injecting into the veins of an animal an antiserum from an animal of a different species which has been induced to form antibody against the kidney substance of the species to which the recipient animal belongs. This observation has been amply confirmed and extended. While the procedure, of course, can have no counterpart in the pathogenesis of any case of human glomerulonephritis, it is not impossible that antikidney antibodies, generated in the human body by other processes, may play a rôle in the pathogenesis of some cases of glomerulonephritis.

In 1943, Rich and Gregory (9), in the course of studies on the experimental production of periarteritis nodosa by means of anaphylactic hypersensitivity, observed that glomerulonephritis, with glomerular alterations having characteristics of those in human acute glomerulonephritis, occurred in rabbits that were sensitized by the intravenous injection of non-bacterial, non-toxic foreign protein (horse serum). Nephritis occurred in animals that had received several intravenous injections of the antigen, but it was found in those and in subsequent studies in this laboratory (10) (11) (12) that repeated injections of

the antigen, while effective, are not essential for the production of the glomerular lesions, for they occurred in animals given only a single injection of foreign protein under conditions which permit a sufficient amount of the antigen to be present in the blood stream and tissue fluids when antibody formation, stimulated by the presence of the protein, is well under way. This can be accomplished most simply by a single intravenous injection of an ample amount of antigen. During the protracted hypersensitive reaction, accompanied by fever, that begins ordinarily five or six days following the injection (i.e., the occurrence of a condition comparable to human anaphylactic serum sickness produced, following an "incubation period", by a single injection of antigen) glomerular lesions, of varying degree at the time of autopsy, may make their appearance. Hawn and Janeway (13), More and Waugh (14), Wissler, Smull and Lesh (15), Germuth (16), Hamilton and Fremes (17), Mellors, Arias-Stella, Siegel and Pressman (18) and others, using a variety of antigens, have confirmed the occurrence of glomerulonephritis under these conditions, using either one or two injections of the antigen.

In none of our own papers published on this subject, nor in those of others, are the experimental glomerular lesions resulting from hypersensitivity illustrated with sufficient clarity, in comparison with those of human glomerulonephritis, to provide an adequate basis for judgment as to whether the various characteristic lesions of human glomerulonephritis are really reproducible experimentally by means of anaphylactic hypersensitivity. Because of the primary importance of this point, the purpose of the present paper is to attempt to illustrate clearly the basic characteristics of this experimental nephritis in comparison with the lesions of human acute glomerulonephritis, and to consider the relationship between the two from the standpoint of pathology and pathogenesis.

PROCEDURES

The observations to be described are based upon a careful study of the kidneys of 87 rabbits. All were albino males, weighing between 2100 and 3600 grams, with an average weight of about 2500 grams. The animals were kept in separate cages in the same room. They were given an unrestricted amount of Rider's Complete Rabbit Pellets or Purina Rabbit Chow Checkers and water. Some were given a weekly supplement of carrots. No relation between the type of diet and the incidence or severity of the renal lesions was observed.

In all except 7 rabbits, which were given 2 to 4 intravenous injections of serum and were autopsied 19 to 34 days after the first injection, the procedure was to give each animal, in groups of 12 to 20, a single intravenous injection of 10 cc. of sterile horse serum, without preservative*, per kilogram of body weight. The animals were killed on the 14th to 18th day after the injection of the serum. Careful autopsies were performed. The tissues were fixed in Zenker-formol and in 10% formalin. Other experimental details, and effects of this sensitization procedure upon tissues other than the kidney, can be found in the studies from this laboratory mentioned above (9), (10), (11), (12).

* The horse serum was very kindly provided by Sharp and Dohme, Inc.

In the ordinary acute glomerulonephritis of the human being a striking early alteration in the glomerular tuft is an increase in the number and size of cells with round or oval nuclei (Figs. 2 and 12). There has been a considerable discussion and divergence of opinion regarding the nature of these cells, i.e., whether they are endothelial, epithelial, interstitial connective tissue cells, mononuclear phagocytes, or a mixture of cells of these types. Most students of the question today believe that while all of these cells may participate in the reaction as it runs its course, the increase in cellularity in the early stages of the process is due mainly to the proliferation and swelling of the endothelial and epithelial cells of the tuft, and to the accumulation of a variable number of leucocytes in the tuft. The divergent views regarding the cells responsible for the increased cellularity of the tuft in acute nephritis are attributable to the fact that, in the usual preparation, it is peculiarly difficult and for the most part impossible to determine with clarity the nature of the individual cells in the cell-rich affected tufts. This is no less true of the experimental nephritis resulting from hypersensitivity. The problem is at present being studied, in collaboration with Dr. Stanton L. Eversole, under conditions which, it is hoped, may yield more certain information about the matter.

The cell-rich glomeruli in acute glomerulonephritis appear more or less ischaemic (Figs. 2 and 12). This seems to be due primarily to impairment of blood flow through the glomerulus, resulting from encroachment upon the capillary lumina by the swelling and proliferation of the endothelial cells. The damaged endothelium permits the escape of protein and even red blood cells into Bowman's capsule and thence into the tubules (Fig. 15), and polymorphonuclear leucocytes, which are often present in increased numbers, migrate from the tuft capillaries. If hemorrhage or exudate clots in the capsular space, the capsular epithelium may proliferate into and over it, resulting in the formation of cellular masses between the tuft and the capsule,—the so-called "epithelial crescents" (Fig. 17). Coagulated exudate may bind portions of the tuft to the capsule, and such adhesions may become invaded by fibroblasts and covered by epithelium (Fig. 19). Hemorrhage or exudate that escapes into the substance of the tuft, or clots between the capillary loops of the tuft, may likewise become organized, together with the damaged tuft structures, permanently solidifying and destroying the function of a portion or all of a glomerulus (Fig. 21). If the damage to a glomerulus is not sufficient or of the character to lead to organization, and the patient survives, the glomerulus eventually returns to its normal state both morphologically and functionally, as is evident from autopsies on individuals who die from unrelated causes after a lapse of time following an attack of acute glomerulonephritis.

These are the familiar characteristics of the glomerular alterations in human acute glomerulonephritis, and all of these glomerular alterations occur in the experimental nephritis. Of the 87 animals that were subjected to the serum

sickness type of protracted anaphylactic reaction in the present study, 53 (61%) developed one or more of these types of glomerular lesions. The lesions were of marked degree in 16 (18.4%) of the animals, of moderate degree in 14 (16%), and were slight but definite in 23 (26.4%). A comparison of the illustrations of this experimental anaphylactic glomerulonephritis (Figs. 4, 5, 6, 7, 8, 9, 16, 18, 20, 22) with those illustrating the typical lesions of human glomerulonephritis (Figs. 2, 12, 17, 19, 21) will make it clear that all of the glomerular alterations that are characteristic of human glomerulonephritis can result from an anaphylactic hypersensitive reaction produced by antigens that have no primary toxicity.

DISCUSSION

The introduction to this paper mentions observations which, for many years, have suggested that hypersensitivity may be an important pathogenetic factor in many cases of human glomerulonephritis. The experiments here illustrated demonstrate that all of the familiar glomerular alterations characteristic of the ordinary type of human acute glomerulonephritis (glomerular cellular proliferation; hemorrhage; the formation of "epithelial crescents" and of adhesions between segments of the tuft or between the tuft and Bowman's capsule; organization and obliteration of the normal structure of part or all of severely affected glomeruli in the healing process) can result from a simple, protracted anaphylactic reaction produced by antigens that have no primary toxicity. The experimental glomerular lesions can reproduce those of human acute glomerulonephritis with remarkable fidelity, as the accompanying illustrations show. All degrees of the lesions occur in the experimental animal and, when sufficiently developed, they are accompanied by the appearance of albumin, red cells and casts in the urine, and elevation of the blood non-protein nitrogen (19), (20), (21). Hamilton-Paterson and Henderson (21) describe death in coma in two animals, with terminal blood urea of 161 and 400 mg. per 100 cc. respectively. The nephritis in these animals had been produced by repeated injections of horse serum over a period of five or six months.

There are at present no adequate studies of the blood pressure in this experimental nephritis. There are, of course, many cases of clinically outspoken acute diffuse glomerulonephritis without hypertension (22) (23). In our experience, in most of the instances of the experimental nephritis the glomerular capillary obstruction is not severe and extensive enough to warrant one to expect either hypertension or the retention of non-protein nitrogen, and this appears to be true of human acute glomerulonephritis following tonsilitis or scarlet fever, for apart from the urinary abnormalities that may occur during the height of the fever, as in other infections, studies in cases of streptococcal tonsilitis and scarlet fever have shown that protein, blood cells and casts frequently appear in the urine after a latent period of a week or more following the onset, in the

absence of any other laboratory or clinical evidences of renal involvement (24) (33). Similarly, in the experimental nephritis, Waugh and More (19) found that albumin, red cells and casts first appear in the urine after a latent period of a week or more following the intravenous injection of foreign protein. This latent period between the injection of the antigen and the appearance of the anatomical and functional abnormalities corresponds to the period during which an adequate amount of antibody is being formed, and it offers at least strongly suggestive support for the view that the well-known latent period between the onset of tonsilitis or scarlet fever and the appearance of urinary abnormalities and frank clinical acute glomerulonephritis may represent the period during which sensitizing antibody is being formed.

It may be mentioned that glomerular alterations of the type characteristic of "subacute" glomerulonephritis (epithelial crescents, adhesions, organization of the damaged tuft) can develop quite rapidly when conditions are suitable. In some human cases in which death has occurred from clinically typical acute nephritis one may find some crescents and adhesions and even badly disorganized glomeruli (Figs. 17, 19, 21); and Figs. 18, 20 and 22 illustrate lesions of this type in the kidneys of a rabbit that was killed on the 15th day after a single intravenous injection of horse serum. The advanced state of these lesions leaves no doubt that they would have been present had the animal been killed several days earlier. Other animals of the series, treated in the same manner and killed at the same time, showed only swelling and proliferation of the cells of the glomerular tuft or no lesions at all. The differences are doubtless due to differences in the rapidity and degree of antibody formation in different individual animals and, in all probability, to additional individual differences which are mal-understood at present. There must be few, indeed, who have worked extensively with this experimental nephritis who have not been impressed by the great importance of these individual and unpredictable differences. They are, with little doubt, responsible for the failure of occasional investigators to produce adhesions or crescents (25), or even any glomerular lesions at all (21), by a single injection of foreign protein.

Polymorphonuclear leucocytes are ordinarily less numerous in the experimental glomerular lesions than in many cases of human acute glomerulonephritis, though occasionally they are plentifully present. This has been noted by Mellors, also (18). The presence of large numbers of polymorphonuclear leucocytes is by no means a constant finding in human acute glomerulonephritis. In the autopsy files of this Department there are numerous cases of classical clinical and pathological acute diffuse glomerulonephritis in which the glomeruli show the typical marked increase in cellularity, and in which polymorphonuclear leucocytes are very scanty.

Hamilton-Paterson and Henderson (21), from their study, state that the experimental nephritis produced by hypersensitivity differs from human acute

glomerulonephritis in that "in human acute nephritis the cellular proliferation of the tuft is more marked". Figs. 4, 5, 6, 7, 8 and 9 of the present paper show clearly that the glomerular cellular proliferation in the experimental lesions can be altogether as marked as in human glomerulonephritis (Figs. 2 and 12). In this, as in the case of the other characteristics, there can be marked individual differences in different animals, as has been mentioned above, and the same is true of the human being. There are differences in the degree of proliferation even in fatal cases, and the many instances in which, after a latent period following scarlet fever or tonsilitis, protein, blood cells and casts appear in the urine in the absence of any other clinical or laboratory evidences of acute nephritis are, with little doubt, instances in which the glomerular alterations of acute glomerulonephritis are much less marked than in the cases seen at autopsy, in which the patients died from renal insufficiency.

While all of the types of glomerular lesions that occur in ordinary human acute glomerulonephritis can be faithfully reproduced in the experimental animal by a hypersensitive reaction, as the accompanying illustrations show, it is exceptional in the experimental animal that the glomerular involvement is as uniform throughout the kidney as it is in most cases of *fatal* acute glomerulonephritis in the human being. It must be remembered, however, that the acute experimental lesions are not derived from animals that have died as a result of severe glomerulonephritis. They are therefore analogous to the ordinarily transient glomerular damage that so commonly follows streptococcal infections in the human being and is manifested, as in the experimental animal, by the appearance of protein, blood cells and casts in the urine without clinical symptoms—a degree of glomerulonephritis obviously less severe or extensive than the fatal nephritis from which the pathological picture of "typical" acute glomerulonephritis has been constructed. Since patients do not die in the early, symptomless stage of severe acute glomerulonephritis, or from the common mild glomerulonephritis manifested only by transient urinary abnormalities, we are not well instructed regarding the character and extent of the glomerular lesions in these conditions; but since the clinical and laboratory manifestations in different cases shade gradually in severity into outspoken acute glomerulonephritis, it is reasonable to believe that the milder stages represent less marked and extensive glomerular lesions of the same basic type as those in fatal acute glomerulonephritis.

In the experimental nephritis, even when the tufts of most of the glomeruli are diffusely and uniformly involved in the proliferative process, as in Figs. 4, 5, 6, 7, 8 and 9, in other glomeruli only portions of the tuft may be involved, or in one part of the tuft there may be a marked, focal accentuation of the proliferative process that is more uniform elsewhere in the tuft (Fig. 13). It is pertinent that this focal accentuation of proliferation in a diffusely involved tuft occurs occasionally in human acute glomerulonephritis also (Fig. 12).

Hamilton-Paterson and Henderson (21), from experiments in which foreign protein was repeatedly injected intravenously for periods up to seven months, suggested that the experimental nephritis produced by hypersensitivity is more comparable to a type of nephritis that occurs in some cases of periarteritis nodosa in man than to ordinary acute glomerulonephritis. The nephritis associated with periarteritis nodosa in man may be either focal or diffuse. In different cases, or even in the same case, glomeruli may show cellular proliferation ranging in degree from marked to slight or none at all; focal necrosis of the tuft is common, and in some instances necrosis of the entire tuft occurs; and a deposition of eosinophilic material, either focally or diffusely in the tuft, is common (Figs. 23, 26). All of this can occur in the experimental nephritis produced by hypersensitivity, but the usual glomerular lesion in the acute experimental nephritis is characterized by cellular proliferation of the tuft, either diffusely throughout the tuft as in human acute glomerulonephritis (Figs. 4, 5, 6, 7, 8, 9), or concentrated focally (Fig. 12). Focal necrosis of the tuft and focal deposition of eosinophilic material, comparable to the alterations in the tuft in the nephritis associated with periarteritis nodosa in man, can be produced experimentally by repeated injections of the antigen (the procedure used by Hamilton-Paterson and Henderson (21)), but it is very rarely encountered following a single injection (Figs. 14, 24 and 25), which produces, rather, the proliferative type of lesion. The experimental nephritis, therefore, is important in showing that hypersensitivity can cause not only the focal necrosis of the glomerular tuft that characterizes the nephritis associated with periarteritis nodosa in the human being (and it is well established that periarteritis nodosa can be caused by hypersensitivity (27)), but also the glomerular alterations that are characteristic of the fatal acute glomerulonephritis that follows streptococcal infection in man. Which type of lesion the hypersensitivity reaction will produce in a given glomerulus is governed by conditions that are at present entirely obscure. This is somewhat analogous to the fact that, in the human being, the hypersensitive reaction to a given antigen may be manifested in the skin by urticaria, or purpura, or exfoliative dermatitis, or eczema, or any one of a variety of other cutaneous eruptions, and in some cases by a combination of two of these types of lesions. Furthermore, comparable in a sense to the focal and the diffuse involvement of the kidney in nephritis, in one case only a few cutaneous lesions may develop, while in another myriads will appear all over the body. The factors that determine which type of cutaneous lesion will occur, and how diffuse the lesions will be, are as obscure as those that determine the type and the distribution of the glomerular lesions in the nephritis produced by hypersensitivity.

In relation to focal glomerulonephritis, it may be mentioned that focal lesions of the glomerular tuft, resembling those of the so-called "focal embolic nephritis" associated with subacute bacterial endocarditis, sometimes occur in

the experimental nephritis produced by hypersensitivity, though only rarely in the acute nephritis produced by a single intravenous injection of the antigen (Figs. 24 and 25). There need be no doubt that embolization of glomerular capillaries by dislodged bits of valvular vegetations occurs in subacute bacterial endocarditis; but there are reasons to question whether all of the focal glomerular lesions in such cases are the results of embolization, and indeed, this question has often been raised. Forty years ago, Longcope (42) suggested that these lesions are not embolic, but the results of hypersensitivity; and that has been repeatedly suggested since (43). It is of interest that focal glomerular lesions quite like those in cases of subacute bacterial endocarditis occur in man in association with periarteritis nodosa (Fig. 26). Ralston and Kvale (44), for example, designate these lesions in cases of periarteritis nodosa as the "embolic" type. Their occurrence in cases of periarteritis nodosa may suggest that those that occur in association with subacute bacterial endocarditis are results of hypersensitivity, not of emboli. In the interpretation of lesions of this type found in cases of periarteritis nodosa, however, it should be borne in mind that glomerular capillaries can readily be embolized by bits of thrombi from intrarenal arteries affected by periarteritis nodosa, when such are present. The experimental production of similar lesions by means of hypersensitivity (and in the absence of periarteritis nodosa of the renal vessels) lends substance to the view that hypersensitivity can produce them in man, but it would be decidedly unwise to conclude that emboli cannot produce them also.

Much remains to be learned regarding the *mechanism* by which the experimental glomerulonephritis produced by hypersensitivity operates. From the beginning it was reasonable to believe that the renal lesions, like those of the cardiovascular system (periarteritis nodosa and cardiac lesions) which can be produced by the same procedure (27), are results of hypersensitivity,—i.e., a tissue injury produced by an antigen-antibody reaction. There is now strong supporting evidence for this view. In the first place, all of our animals that developed nephritis were demonstrably hypersensitive, often in a very high degree, as shown by the skin test. In the second place, it is known that focal reactions of hypersensitivity can produce in other parts of the body capillary lesions (e.g., urticaria, purpura) of the same type as those in the glomeruli in the experimental nephritis. Regarding the proliferation of endothelial cells, the anaphylactic hypersensitive reaction can incite an extraordinary degree of focal endothelial proliferation even in arteries, as is seen in pariarteritis nodosa (9).

In the third place, there is a latent period between the injection of the antigen and the appearance of the glomerular lesions,—a period that corresponds to that during which a sufficient amount of antibody is being formed to clear the excess of injected antigen rapidly from the circulation. Hawn and Janeway (13), using purified antigens, were the first to show the correlation between the

rate of disappearance of the intravenously injected antigen from the blood (which is determined largely by the rate and degree of antibody formation), the rise in antibody titre, and the appearance of the renal lesions. Their important observations, which were made with only a three-tube antigen-dilution precipitin test for antigen and a very simple one-tube ring test for antibody, were firmly established as correct by the detailed quantitative studies of Germuth (16) (26),—studies which have rendered our information more precise.

That the renal lesions are the result of an antigen-antibody reaction is further supported by Germuth's (26) report that in animals that had been sensitized previous to the injection of the nephritis-producing dose of antigen, the antigen was cleared from the blood stream earlier and free antibody appeared earlier in the serum than in normal animals given the same dose, and the time of occurrence of the renal lesions was correspondingly accelerated.

Additional suggestive evidence for the rôle of an antigen-antibody reaction in the production of the experimental nephritis consists in the demonstration by Schwab and his co-workers (28) and Moll and Hawn (29) that a decrease in serum complement occurs during the period when the renal lesions begin to appear, i.e., the period during which antigen is rapidly disappearing from the circulation because of active antibody production. A decrease in the complement titre of the serum in human glomerulonephritis has been reported by numerous investigators (34) (35) (41). While the rôle of complement in anaphylactic hypersensitivity has not yet been clarified, the participation of complement in other antigen-antibody reactions renders these observations pertinent.

Finally, Schwab and his co-workers (28) have shown that in animals in which the ability to produce antibody is suppressed by x-radiation or nitrogen mustard, no fall in complement titre occurs following the injection of the antigen, and renal lesions do not develop. While x-radiation and nitrogen mustards produce bodily effects other than suppression of antibody formation, effects which in themselves might possibly prevent the development of the renal lesions, the experiments are nevertheless highly suggestive.

The combined evidence that the experimental nephritis is the result of an antigen antibody reaction is therefore very strong. There is lacking only the demonstration that the lesions can be promptly produced by the passive transfer of antibody in the presence of the antigen. Difficulties attend this demonstration, but it is probable that they can be overcome.

Unfortunately, the mechanism by which an antigen-antibody reaction injures tissue is still completely obscure. This constitutes a problem of urgent importance in all of the manifold types of injury produced by hypersensitivity. Apart from the obscurity surrounding the actual process by which the interaction of antibody with antigen produces tissue damage, it is not clear whether the antigen must be first fixed to the tissues and the antibody then react with

it, or *vice versa*. There have been proponents of each of these views. Since the prompt wheal that is the characteristic reaction to the injection of the antigen into the skin of the anaphylactically sensitized body, can be produced in the normal body either by injecting an antigen into the skin and following it by the injection of its antibody at the same site, or by injecting the antibody first and following it by the antigen, it appears unnecessary to assume that the reaction demands the preliminary local fixation of a particular one of the two components. If a preliminary fixation is necessary at all it would appear that either the antigen or the antibody can serve. However, since antigens and antibodies that enter the circulation soon permeate the tissues throughout the body extravascularly, no actual local fixation to the tissues may be essential for the hypersensitive reaction, but only a meeting of antigen and antibody at the proper site under the proper conditions. Further investigation is required for the clarification of even this primary point. Mellors and his co-workers (18), using a microfluorescence method for the detection of antibody in tissue sections, found a greater amount of antibody in the damaged glomeruli of the acute glomerulonephritis produced by hypersensitivity than in normal glomeruli. From this they concluded that "this localization of antibody, which is presumably due to a previous fixation in the glomeruli of some of the injected antigen, is an essential requisite for the allergic pathogenesis of glomerulonephritis". In the interpretation of studies of this nature, it is important to remember that it is well established that circulating antibodies, as well as foreign proteins, colloidal dyes, filterable viruses and even particulate matter and bacteria, escape with the exudate at inflamed sites and become concentrated there (38) (39) (40). A greater concentration of antibody in the inflamed glomeruli of glomerulonephritis may, therefore, be the ***result*** of the glomerular damage, not the cause. While there is, of course, no reasonable doubt that antibody is required for the glomerular damage caused by hypersensitivity, control studies on the concentration of antibody in non-specifically inflamed glomeruli in sensitized animals are necessary for the satisfactory interpretation of the amount of antibody shown by the fluorescence method in the inflamed glomeruli in the above-mentioned experiments.

While there is every reason to believe that a proper balance between antibody and antigen plays an essential rôle in the pathogenesis of the experimental nephritis, it is altogether unlikely that the occurrence of the nephritis, or its severity, depends solely upon the amount of antigen in the circulation or tissues and the amount or rate of antibody production. There is persuasive evidence that other factors can play a determining rôle in the development of lesions resulting from hypersensitivity. Thus, although Germuth (16) has shown that the peak incidence of glomerulonephritis and of periarteritis nodosa occur at the same period of time following a single injection of antigen, and while both lesions often occur together, our own early (9) and subsequent

studies, and those of others (13) (16) (17) (20), have shown that individual animals may develop glomerulonephritis and not periarteritis nodosa or *vice versa*. In the human being, likewise, widespread periarteritis nodosa resulting from hypersensitivity to drugs, for example, often occurs in the complete absence of glomerular lesions, while in other individuals sensitized to the same drug both occur together. Indeed, the fact that even in the same kidney some glomeruli may be markedly affected while others are less so or remain entirely normal, indicates that local factors can influence the development of the lesions independently of the amount of antigen introduced into the circulation or the amount of antibody formed.

It is highly likely that in addition to the well-known individual differences in the ability to produce antibody, and apart from local tissue factors, other more generalized, ***non-specific bodily conditions*** may also influence the occurrence or severity of lesions produced by antigen-antibody reactions, and so play an important rôle in determining the familiar differences in the reaction of different individuals. Pertinent in this regard, it was shown in our laboratory (10) (11), and amply confirmed by others, that cortisone and ACTH can markedly inhibit the development of anaphylactic nephritis and periarteritis nodosa. In our experiments no significant difference was found between the antibody titres of the hormone-treated animals that did not develop the lesions and those of the controls that did. However, a complex antigenic stimulus (horse serum) and the antigen-dilution precipitin test for antibody were used; and while those experimental tools have served to yield basically important information regarding many fundamental antibody relationships, which subsequent more precise methods have confirmed, Germuth (16) (30) has quite properly re-emphasized their limitations. Using an immunologically homogeneous antigen and a more precisely quantitative test for antibody, Germuth (30) found that cortisone, administered throughout the experiment from the beginning of sensitization, inhibited antibody formation and the Arthus phenomenon (the local injury produced by the injection of the antigen into the skin of the highly sensitized body). ACTH was less effective. If treatment with cortisone was postponed until two weeks after sensitization was begun, circulating antibody was reduced in comparison with untreated sensitized controls, but not sufficiently, in animals with a high antibody titre, to affect the intensity of the Arthus reaction. Furthermore, treatment with cortisone did not appreciably reduce the antibody titre or the intensity of the Arthus reaction in passively sensitized animals. Germuth, therefore, regarded his studies as indicating that the vascular damage in hypersensitive reactions is suppressed by cortisone as a result of the hormone's inhibition of antibody formation, rather than by its non-specific effect which, as is well known, decreases vascular reactivity to other types of injurious agents.

In Germuth's clear-cut and beautifully documented experiments, the obser-

vations on tissue damage produced by hypersensitivity were limited to the study of the cutaneous Arthur phenomenon. Wedgewood, Hawn and Janeway (36), on the other hand, in a study of the nephritis produced by hypersensitivity, found that if treatment with ACTH was delayed until 5 days after the first of two intravenous injections of antigen, in order to provide time for the initiation of antibody formation before hormonal treatment was begun, the rate of disappearance of antigen from the circulation (which is determined by the rate and amount of antibody production), the fall in complement (a further evidence of antigen-antibody reaction) and the appearance of free antibody, were indistinguishable from those events in the sensitized controls that were not given ACTH and which developed nephritis; but no renal lesions developed in the ACTH-treated animals. Wedgewood and his co-workers, therefore, could not attribute the suppression of the glomerulonephritis to suppression of antibody production with a consequent absence of the injurious antigen-antibody reaction, but regarded it rather as the result of the action of ACTH upon the tissues, altering their capacity to react to the irritant effects of the antigen-antibody interaction. This conclusion had been reached in our own previous studies (10), in which less precise methods were used, and there is persuasive additional evidence to support its validity. There is much evidence that cortisone acts non-specifically to render the capillary endothelium less reactive to injurious agents in general.

In the first place, it is now well established that ACTH and cortisone suppress inflammation and also capillary endothelial proliferation (31) (32) incited not only by antigen-antibody reactions, but by the widest variety of injurious agents which have nothing to do with an antigen-antibody reaction. It is quite clear that the action of cortisone in rendering capillaries resistant to stimuli that incite inflammation and endothelial proliferation is a general one, and not specific for this or that type of injurious agent. Furthermore, it has been shown by Woods (45), Woods and Wood (46) and others that cortisone inhibits the vascular response to the irritant effect of antigen-antibody reactions even when the hormone is applied locally at the site of injury, and in small amounts that could not possibly affect antibody production or the level of antibody in the circulating blood. Finally, it is well established clinically that in anaphylactic urticaria cortisone often suppresses the development of new wheals with a promptness that virtually precludes any effective reduction in the existing level of circulating antibody. Purpura, which also can result from anaphylactic hypersensitivity, is likewise suppressed by cortisone. Since the glomerular capillary lesions of the nephritis of hypersensitivity may, in effect, be regarded as miniature analogues of cutaneous urticaria and purpura, it is not surprising that they, too, can be prevented by cortisone even though an effective amount of antibody is produced.

That in the experimental animal cortisone may not suppress the anaphylactic

Arthus reaction as effectively as it suppresses the anaphylactic visceral cardiovascular and renal lesions in the same body (10) is analogous to the prompt and effective suppression of anaphylactic asthma by cortisone in the human being, while in the same body the skin test may remain positive. The efficacy of the hormone in inhibiting tissue damage produced by injurious agents depends upon the intensity of the stimulus and the amount of hormone administered. The abrupt injection of a relatively large amount of antigen into a tissue site in the sensitized body (the procedure used to elicit the Arthus reaction) is a more violent and extensive stimulus than that produced by the minute amount of antigen which interacts spontaneously with antibody at an individual microscopic internal site, such as the capillaries of a glomerulus in nephritis or a minute focus in a microscopic artery in periarteritis nodosa. In the Arthus reaction, therefore, an intense and macroscopically extensive reaction is produced, manifested by a large area of edema, erythema and hemorrhage, often with attendant necrosis, in contrast to the minute, microscopic glomerular capillary damage produced by the antigen-antibody reaction. That a given dose of cortisone may suppress the latter damage but not the former is not surprising, and has its counterpart in the different degrees of efficacy of a given amount of the hormone in suppressing the reaction to stimuli of different intensity produced by other types of injurious agents.

In the hands of most investigators, cortisone has had no significant therapeutic effect upon acute glomerulonephritis in the human being. In the experimental studies on animals, in which cortisone has suppressed the development of glomerulonephritis, the hormone has been administered during the period of sensitization *before* the nephritis had developed, and its effect has been to prevent the development of the nephritis. In the human being, in which the hormone has been administered only after the glomerular damage has been completed and has become clinically evident, it has had no appreciable effect upon the condition and would hardly be expected to have had.

The above studies on the effect of cortisone on reactions of hypersensitivity were mentioned only as an example of a systemic influence which can profoundly affect the individual tendency to develop the glomerulonephritis of hypersensitivity. What the factors are which determine the marked, native individual differences in susceptibility to the development of glomerulonephritis in the experimental animal (and in man) is totally obscure, as are the factors that determine whether a person who becomes sensitized to a particular antigen will suffer any injurious effect from natural contact with the antigen, and if so, what form the injury will assume; for it is well known that in different individuals sensitized to the same food allergen, for example, ingestion of the allergen may produce no effect at all, or such widely different effects as a gastrointestinal upset, vasomotor rhinitis, urticaria, purpura, eczema, or an asthmatic attack. The problem of the mechanism of native indi-

vidual resistance or susceptibility to sensitizing agents is as deplorably obscure as that relating to native individual resistance and susceptibility to infectious agents, and as pressingly important. Individual differences in the ability to produce antibodies is by no means the sole factor concerned.

Evidence has been presented that all of the types of glomerular lesions that characterize human acute glomerulonephritis, together with the associated urinary abnormalities, can be produced in the experimental animal by hypersensitivity to non-toxic foreign proteins, and that when the experimental lesions are severe enough death in uremia occurs. What, now, is the significance of this experimental nephritis for the problem of the pathogenesis of human acute glomerulonephritis? In man, the latent period between the height of a streptococcal infection and the onset of glomerulonephritis is highly suggestive that human acute glomerulonephritis, also, results from an antigen-antibody reaction, which requires a latent period during which an adequate amount of antibody is being formed. The experimental reproduction of the glomerular lesions as a result of hypersensitivity to nontoxic antigens after a latent period following the introduction of the antigen does not, however, prove that hypersensitivity is the mechanism responsible for the injury in human glomerulonephritis. Final proof or disproof of the rôle of hypersensitivity in human glomerulonephritis must rest upon further studies of the problem in man. The complete solution of that problem must explain certain circumstances associated with the human disease.

In the first place, the important studies of Rammelkamp (3) (37) provide strong evidence that some types of group A streptococci (particularly types 12 and 4) are more nephritogenic than others. This might be interpreted as an indication that the nephritogenic types produce a toxic product that is directly injurious to the renal glomeruli and which the other types do not produce. The latent period is, however, difficult to understand if the glomerular injury is a direct toxic one, for the injury would be expected to become manifest during the height of the infection when the toxic product would be liberated in greatest abundance and when the patient is suffering from the systemic toxic effects of the infection. This, however, is not the case. Glomerulonephritis ordinarily appears during convalescence from the infection, after the patient has become afebrile and symptom-free. The latent period cannot be explained as being simply the time required for a glomerular injury occurring during the height of the infection to become clinically evident, for it is to be emphasized that the urinary abnormalities (albumin, blood cells, casts) appear suddenly during convalescence after a latent period during which the urine was clear. This is comparable to the appearance of protein, blood cells and casts in the urine after a latent period of a week or more during which antibody is being formed following the intravenous injection of foreign protein in the experimental nephritis produced by hypersensitivity (19); and the similar situation in human

acute glomerulonephritis is, according to present information, much more understandable as a result of an antigen-antibody reaction than of a direct toxic glomerular injury.

Since there is so much evidence incriminating group A streptococci in the pathogenesis of both acute glomerulonephritis and acute rheumatic fever, the question why there is not a closer correlation between the incidence of the two diseases has long been a perplexing one, particularly since there is also highly suggestive evidence that hypersensitivity plays an important rôle in the pathogenesis of each. Rammelkamp's studies (3) (37) showing that certain types of group A streptococci are more nephritogenic than others offers some help toward the understanding of this problem, for they indicate that only several of the many types of group A streptococci produce glomerulonephritis, while acute rheumatic fever may follow infection with any type. There still remains the problem as to why, if hypersensitivity is a factor in the pathogenesis of both rheumatic fever and glomerulonephritis, some types of streptococci are more nephritogenic than others, though apparently neither more nor less capable of producing rheumatic fever. Nothing is known of the nature of the streptococcal substances which are responsible for the development of either rheumatic fever or glomerulonephritis, and until that is known it will hardly be possible to explain the manner of action of streptococci in the pathogenesis of these diseases. It is possible that the type 12 and type 4 nephritogenic streptococci may produce antigens or haptens that have a greater affinity for glomerular tissues than have the non-nephritogenic types, but that is at present mere speculation. It is, however, known that some antigens that enter the blood stream have a greater tendency to produce lesions of hypersensitivity in one tissue than in another. The ingestion of egg albumin, for example, produces eczema in the sensitized body much more commonly than asthma or arthritis.

Another point requiring explanation is the infrequency of a second attack of acute glomerulonephritis in persons who have recovered from one attack, in contrast to the familiar repeated attacks of acute rheumatic fever. Rammelkamp (3) has suggested that there may be a durable type-specific immunity to group A streptococci produced by infection, and that the rarity of second attacks of acute glomerulonephritis may be simply the result of the paucity of the nephritogenic types, while attacks of acute rheumatic fever may be produced by any of the types. Further studies of the types of streptococci that produce attacks of tonsillitis subsequent to an attack of acute glomerulonephritis will be necessary to clarify this.

SUMMARY

Evidence is presented that all of the types and degrees of glomerular lesions that characterize human acute glomerulonephritis can be produced experi-

mentally by hypersensitivity to non-toxic foreign proteins, i.e., by a tissue injury resulting from an antigen-antibody reaction.

The bearing of this experimental glomerulonephritis upon the pathogenesis of acute glomerulonephritis in the human being is examined and discussed.

Focal necrotizing glomerular lesions resembling those that occur in association with some cases of periarteritis nodosa in man can also be produced experimentally by hypersensitivity to non-toxic foreign antigens.

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EXPLANATION OF ILLUSTRATIONS

(Photographs by Miss Joan Wheaton and Miss Patricia Whitney)

FIG. 1. Normal glomerulus of human kidney, showing normally patent capillaries and normal degree of cellularity.

FIG. 2. Glomerulus from kidney of human acute glomerulonephritis, showing the characteristic marked, diffuse cellular proliferation and relative ischemia.

FIG. 3. Normal glomerulus of rabbit kidney, showing normally patent capillaries and normal degree of cellularity.

FIGS. 4, 5, 6, 7 and 8. Glomeruli of rabbit 71, killed 15 days after a single intravenous injection of 10 cc. of sterile horse serum per kg. of body weight. Note marked, diffuse cellular proliferation and relative ischemia.

FIG. 9. Glomerulus of rabbit 36, killed 17 days after a single intravenous injection of 10 cc. of sterile horse serum per kg. of body weight. Note marked increase in cellularity and relative ischemia.

FIG. 10. Lower magnification of kidney of normal rabbit.

FIG. 11. Kidney of rabbit 71 (see legend of Fig. 4), same magnification as Fig. 10. The increased cellularity of the glomeruli in comparison with the normal (Fig. 10) is shown in their darker and more solid appearance.

FIG. 12. Glomerulus from kidney of clinically typical human acute glomerulonephritis (death 2½ weeks after clinical onset), showing focal accentuation of cellular proliferation at bottom of tuft.

FIG. 13. Glomerulus of rabbit 118, killed 14 days after a single intravenous injection of 10 cc. of sterile horse serum per kg. of body weight, showing focal accentuation of cellular proliferation at left of tuft.

FIG. 14. Glomerulus of rabbit 30, killed 16 days after a single injection of 10 cc. of sterile horse serum per kg. of body weight, showing focal necrosis with beginning repair of the lower part of the tuft. There was periarteritis nodosa elsewhere but none in the renal arteries.

FIG. 15. Hemorrhage in tubule in clinically typical human acute glomerulonephritis (death 1 week after clinical onset).

FIG. 16. Hemorrhage in tubule of rabbit 16, killed 17 days after a single intravenous injection of 10 cc. of sterile horse serum per kg. of body weight. The hemorrhage becomes a hemoglobin cast on right.

FIG. 17. Glomerulus from kidney of clinically typical human acute glomerulonephritis

(death 2½ weeks after clinical onset), showing epithelial crescent in Bowman's capsule on right.

FIG. 18. Glomerulus of rabbit 50, killed 15 days after a single intravenous injection of 10 cc. of sterile horse serum per kg. of body weight. Epithelial crescent in Bowman's capsule on right.

FIG. 19. Glomerulus from kidney of clinically typical acute glomerulonephritis (death 2½ weeks after clinical onset), showing adhesion binding tuft to Bowman's capsule.

FIG. 20. Glomerulus of rabbit of Fig. 18, showing adhesion binding tuft to Bowman's capsule.

FIG. 21. Glomerulus from kidney of clinically typical human acute glomerulonephritis (death 2½ weeks after clinical onset), showing destruction and solidification of tuft and fusion with crescent.

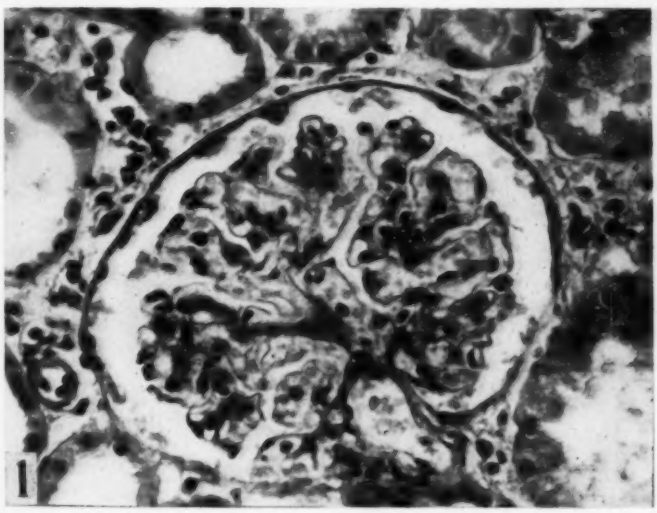
FIG. 22. Glomerulus of rabbit of Fig. 18, showing destruction and solidification of tuft and fusion with crescent.

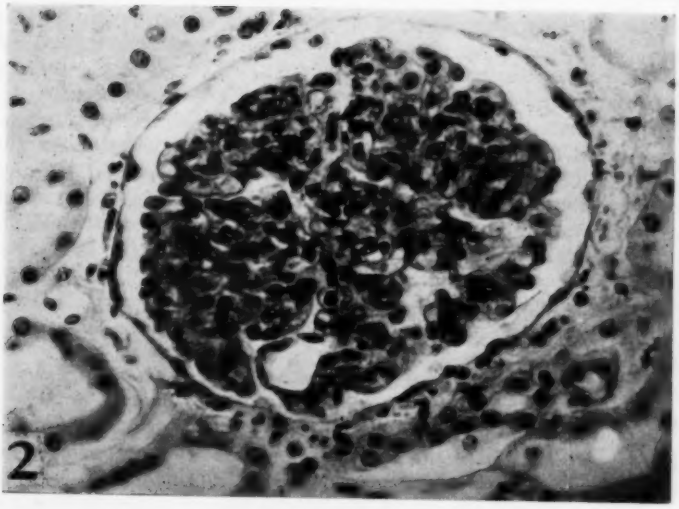
FIG. 23. Glomerulus from human case of periarteritis nodosa, showing eosinophilic deposit in a necrotic focus in tuft. There was periarteritis nodosa of intrarenal arteries, with thrombi in numerous affected arteries. This lesion may therefore have resulted from embolism.

FIG. 24. Glomerulus of rabbit 50, killed 15 days after a single intravenous injection of 10 cc. of sterile horse serum per kg. of body weight, showing eosinophilic deposit in a necrotic focus in tuft. There was periarteritis nodosa in tissues elsewhere, but none in the renal arteries.

FIG. 25. Another glomerulus of rabbit of Fig. 24, showing eosinophilic deposit in a necrotic focus in tuft.

FIG. 26. Glomerulus from human case of periarteritis nodosa, showing a focal eosinophilic deposit in tuft precisely like the "focal embolic" lesions that occur in cases of subacute bacterial endocarditis. There was periarteritis nodosa of intrarenal arteries, with thrombi in numerous affected arteries. This lesion may therefore have resulted from embolism.

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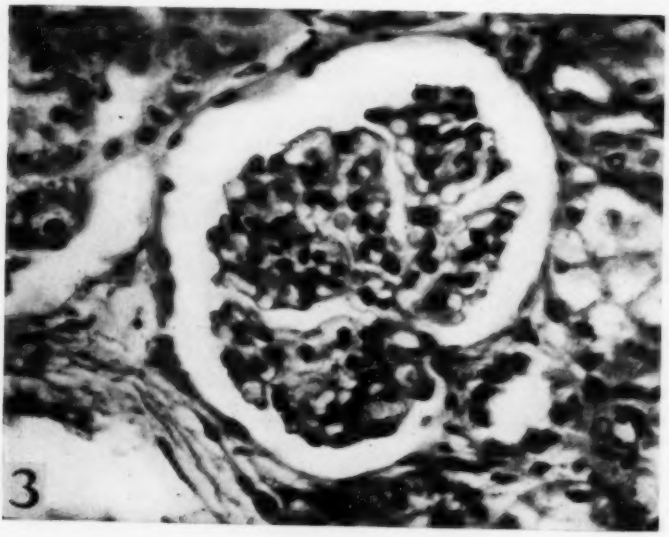
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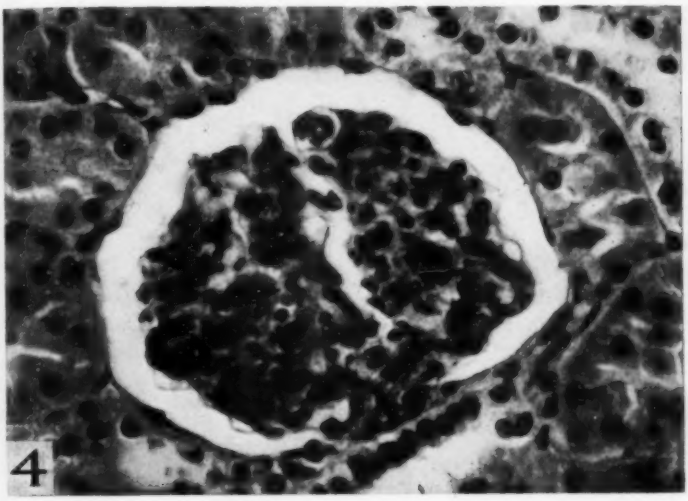
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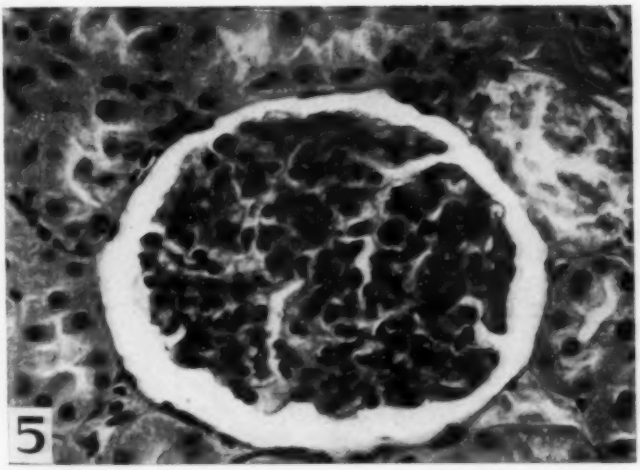
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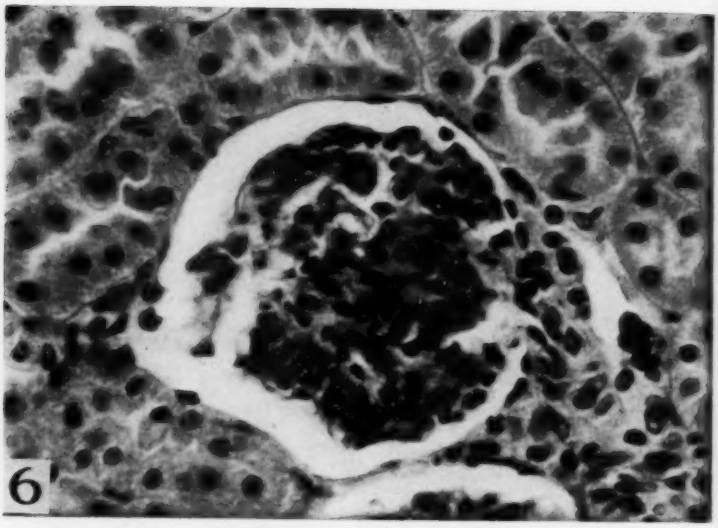

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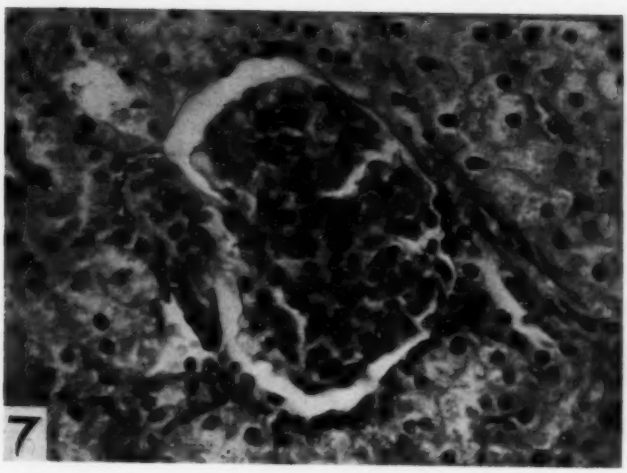

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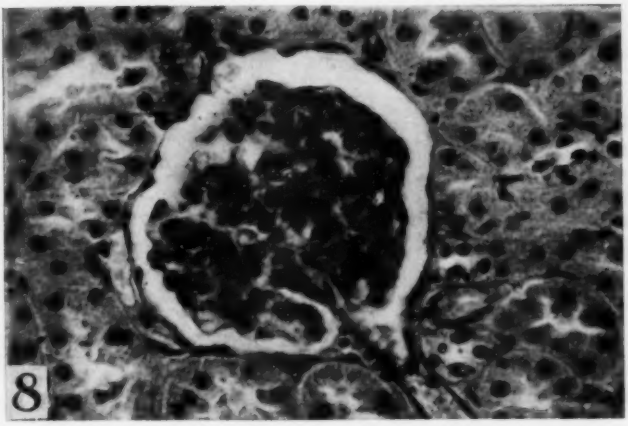
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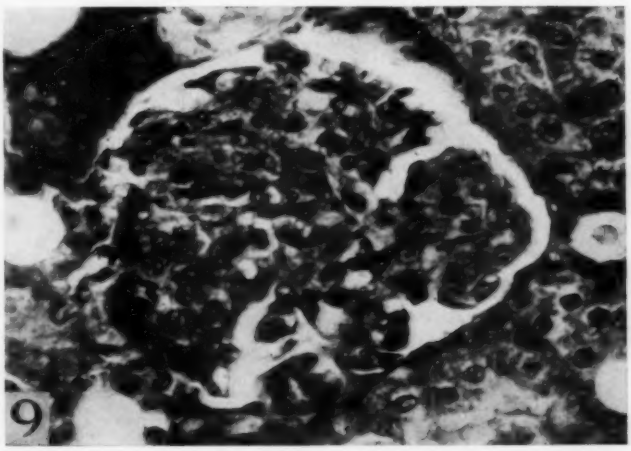
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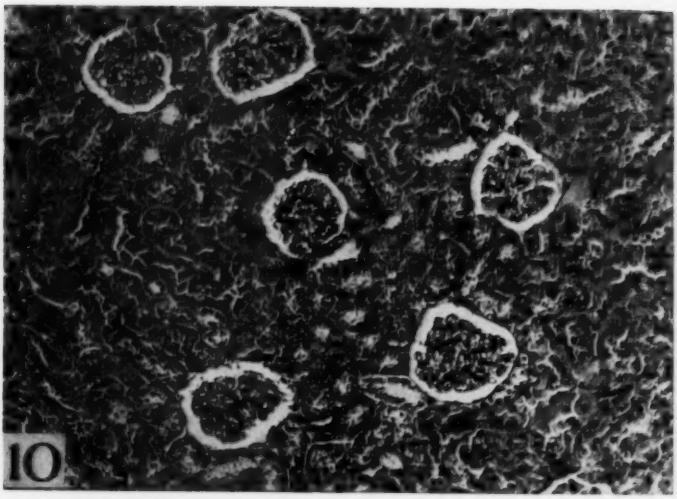
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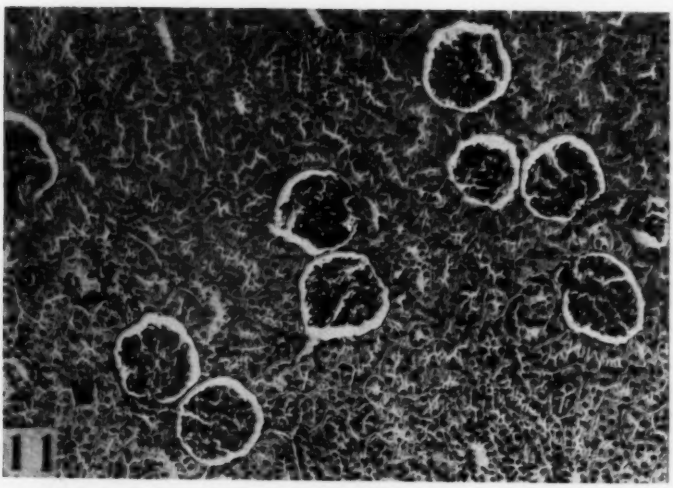
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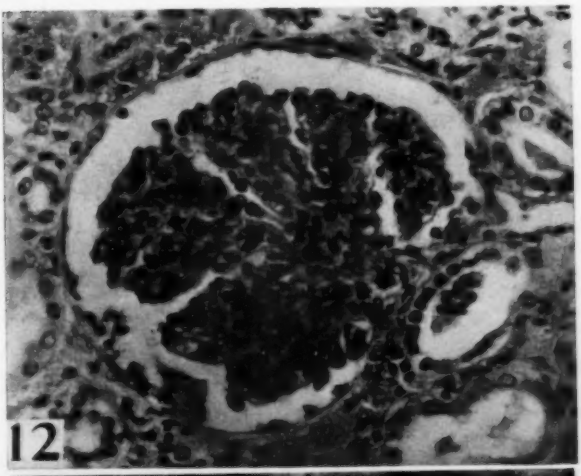

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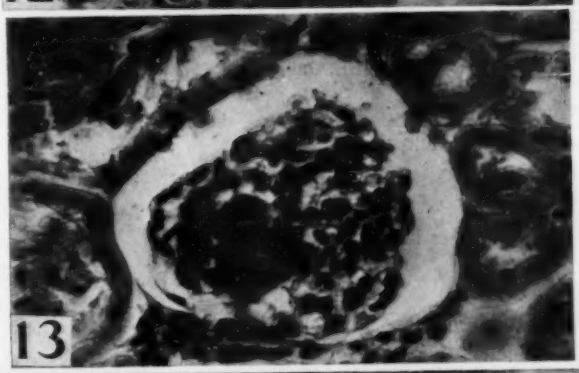

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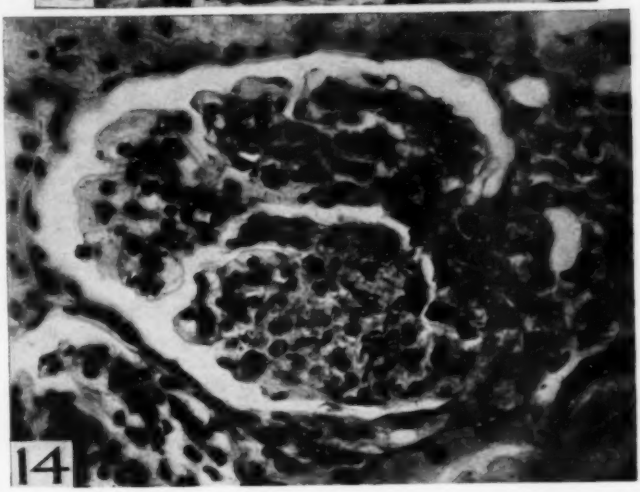

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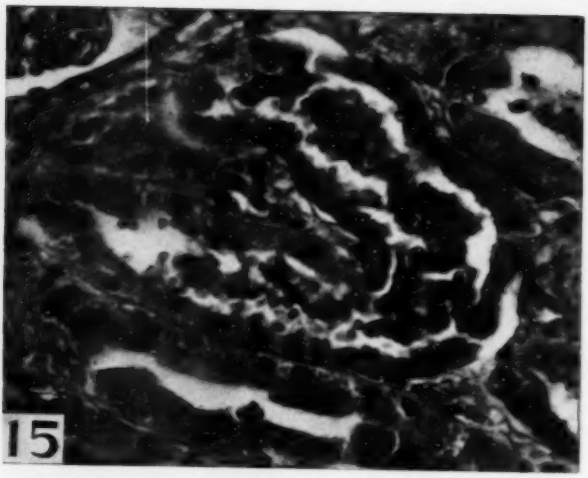

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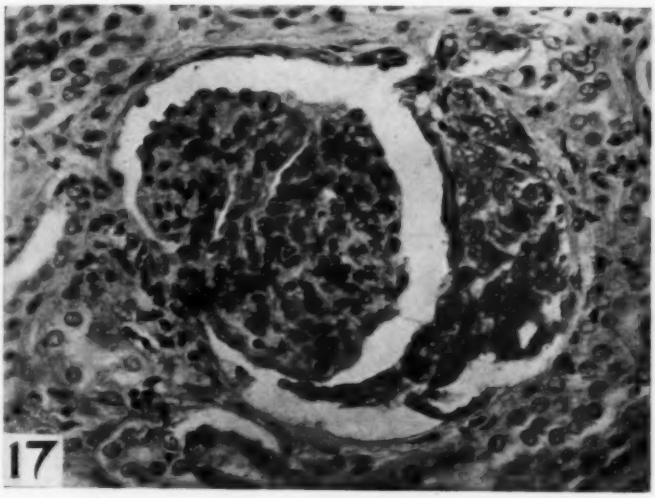

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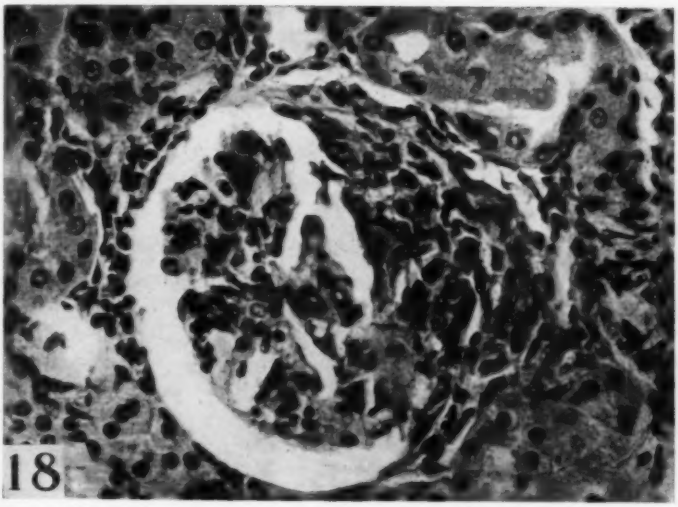

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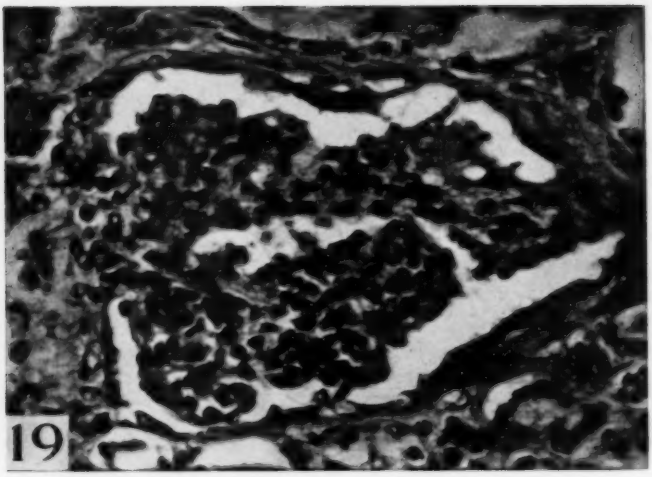
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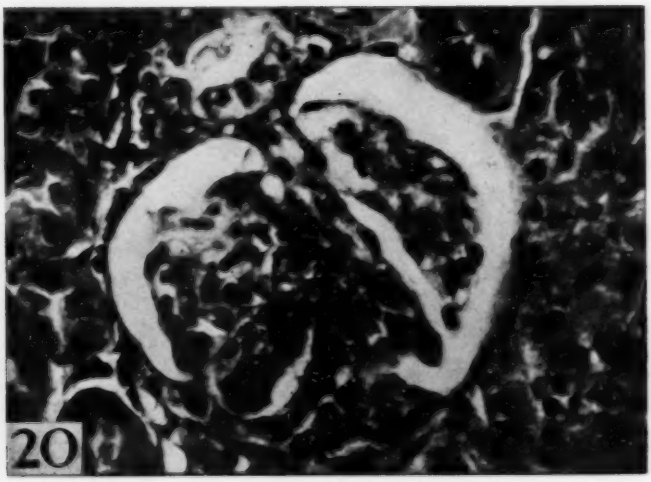
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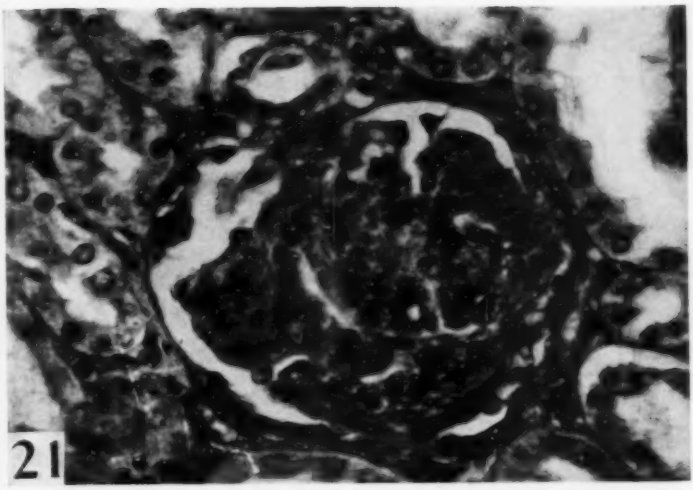

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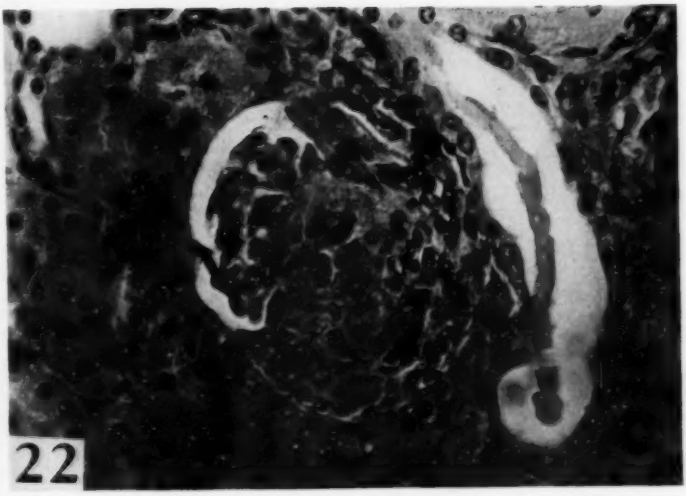

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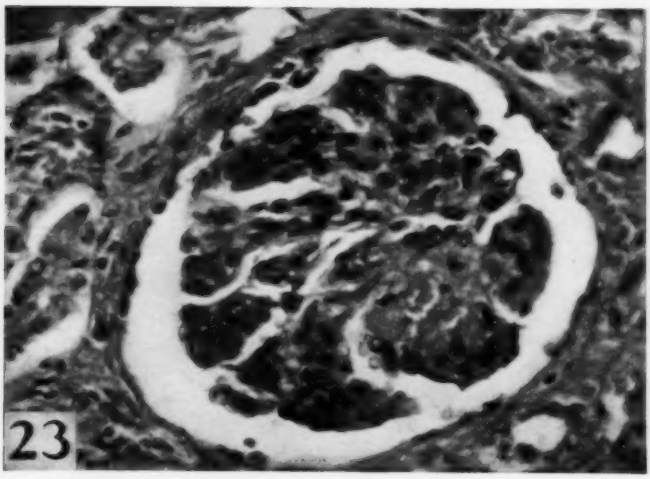

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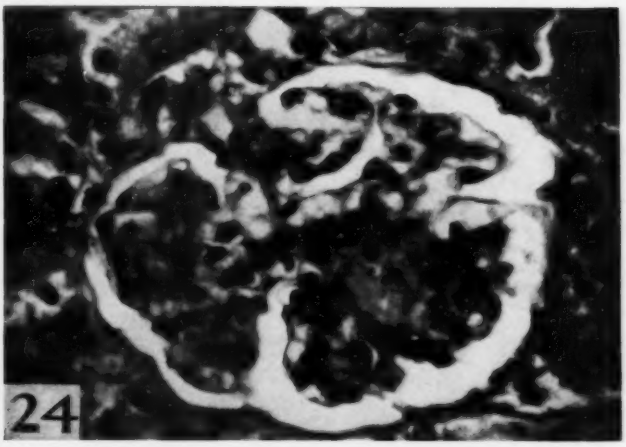

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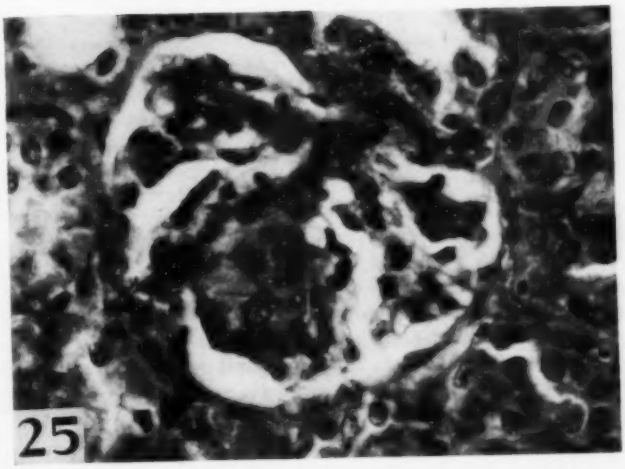

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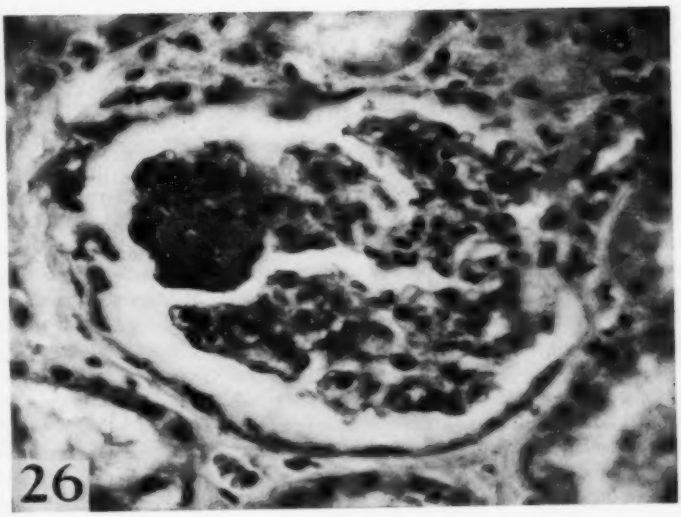


26

ABSTRACTS OF PAPERS PRESENTED BEFORE THE MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

November 14, 1955

THE BALLISTOCARDIOGRAPHIC CIGARETTE TEST

FRANK W. DAVIS, JR., WILLIAM R. SCARBOROUGH, R. E. MASON, M. L. SINGEWALD AND B. M. BAKER, JR.

Because of the need for objectivity in the diagnosis of coronary insufficiency, various efforts have been made by this laboratory to better our currently inadequate methods. The effects of smoking a cigarette on the form of the ballistocardiogram have been studied, because certain patients with coronary artery disease have shown deterioration of pattern with this procedure. The effects of this test on 252 normal subjects and 190 patients with either an old myocardial infarction or angina pectoris have been analyzed. The cigarette test was positive in 7.5 per cent of the control subjects, in contrast to 48.9 per cent of the coronary group. No positive tests occurred in control subjects under the age of 40; positive tests occurred in only 3 of 89 subjects in the 5th decade, and one has subsequently developed a myocardial infarction. Nitroglycerine prevented the deterioration of ballistic forms in the few subjects tested.

It is concluded that a positive ballistocardiographic cigarette test in a subject under the age of 50 suspected of having coronary artery disease might be considered ancillary evidence supportive of this diagnosis. This test is offered more as a challenge to the investigator than as a routine procedure to be used freely in the clinic.

PACEMAKER POTENTIALS IN ISOLATED RABBIT AURICLES EXPOSED TO LOW TEMPERATURE

JEAN M. MARSHALL

The auricles were suspended in a lucite chamber and bathed with physiological saline containing sufficient bicarbonate to give a pH of 7.4 when 2 per cent CO_2 in O_2 was bubbled through the chamber. Action potentials were recorded from the region of the pacemaker in the right auricle and from the tip of the left auricle. The mechanical activity was recorded by allowing the auricles to pull against a muscle lever attached to a transducer tube, RCA 5734. The auricles beat spontaneously. The temperature of the bathing solution was maintained at any given temperature between 10 to 35°C. by thermostatic control within ±0.5°C.

Under these conditions the tissue was cooled from 30°C. to a temperature at which all electrical and mechanical activity ceased. This temperature varied between 14–20°C. As the tissue was rewarmed small, rhythmical, electrical potentials appeared. These potentials exhibited the following properties, (a) they were localized in the region of the pacemaker, (b) they were unaccompanied by measureable tension, (c) they increased in both size and rate as the tissue was rewarmed to a temperature at which they developed into large fully propagated action potentials with the simultaneous development of tension, (d) the addition of acetylcholine (10^{-6} to 10^{-8} g/ml) to the bathing solution at a temperature where these small electrical potentials were the only evidence of activity, caused an immediate appearance of large action potentials and the development of tension, (e) this stimulatory effect of acetylcholine could be potentiated by eserine and inhibited by atropine.

PRESSURE WAVE PROPAGATION IN LIVING HUMAN ARTERIES

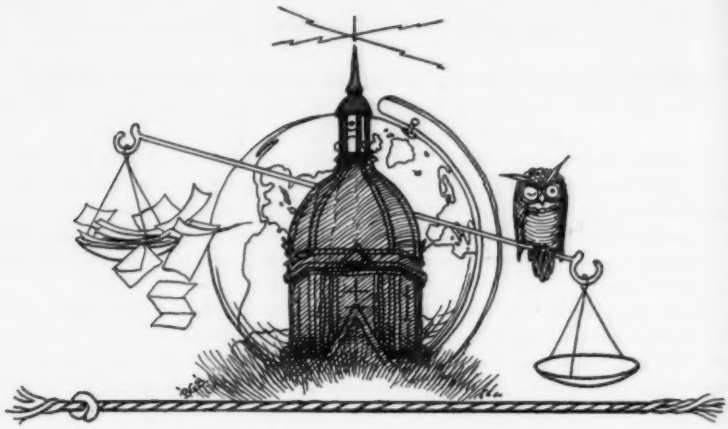
MILTON LANDOWNE

Conventional studies of pressure propagation in living vessels use the wave produced by the heart. This does not permit control of the kind of wave, and little control over the pressure on which the wave rides. A method of creating small pressure waves in an arterial segment, superimposing these upon the ambient continuously varying arterial pressure permits the determination of wave velocity and pressure by accurate intra-arterial pressure recording techniques.

At comparable pressures below 90 mm. Hg, impact wave velocity in the radial artery increases with age until the sixth decade and then tends to decrease. The rise is statistically significant over 20 year spans. At higher pressures an age change is not noted. In contrast to the small changes in velocity with age, the records show the marked dependence of velocity upon pressure. The relation obtained with few exceptions has been a curve concave toward the pressure axis. Average curves were prepared for each of the decade and 20 year age groups, and second order polynomial equations were derived which fit the central portion of these curves to within 1 per cent or better. The curves and fitting equations for the 20 year age groups exhibit a progressive elevation of intercept, decrease in slope, and decrease in curvature toward the pressure axis with age. While these equations are primarily useful as descriptive of the collected information, some interpretations are offered, in a tentative fashion, and with considerable reservation.

If no new factors entered at pressures below our lowest observations (and we feel sure they do), we infer that in its undistended state the artery in the younger subjects would be flaccid and effectively collapsed and not support a wave, while in the older subjects it would remain open, the velocity of propagation increasing with age at low pressures. If the "volume elastic modulus" ($dp/dv/v$) is equal to $1.055 \times \text{velocity}^2$, then the data may be converted into a pressure-volume diagram. For a group of 95 normotensive subjects this suggests a volume change of about 2.5 per cent over the range of pulse pressure. Reasonable dimensional estimates permit deductions as to the stress-strain relationships under these test conditions, and the resulting estimated tangent modulus values increase from 1.2 to 3.4×10^7 dynes/cm.^{2} over the pulse pressure range. A group of 16 hypertensive subjects provide intercept values which are lower than in normotensive subjects of the same or lower age means. The derived pressure-volume curve is displaced toward higher pressures thereby. For small groups of selected younger subjects with clinically marked medial sclerosis the curvature was less than that for mixed subjects of the same or higher age, and in a small group of selected older subjects without clinically evident medial arteriosclerosis the curvature was greater than for sclerotic or unselected subjects of the same age range.

Impact waves travel faster than the conventionally measured pulse wave. Small sinusoidal waves may also be induced, and the velocity of propagation of these waves along the brachioradial artery increases as frequency of the wave is increased. The elastic behavior of this artery is therefore demonstrated to be both frequency and pressure dependent.



BOOK REVIEWS

Introduction to Virology. By GILBERT DALLDORF. 102 pp., $3.50. Charles C. Thomas Co., Springfield, Illinois.

This is an excellent book. It accomplishes its purpose of serving as an introduction to the highly complex field that is modern virology and yet avoids losing the reader in the maze of bewildering and complicated theories and facts that abound in modern virology. Three quarters of the book is given over to brief descriptions of common viruses and virus diseases. Dr. Dalldorf has not tried to include all of the known clinical, pathological, and immunological data in these chapters and yet, by selecting examples carefully and employing an enviable skill for accurate generalization, he has achieved lucidity and efficiency of expression without sacrificing interest or readability of the material. The treatise concludes with a brief outline of the basic facilities and equipment needed for diagnostic or experimental virology and some of the fundamental techniques useful in this field. There are several helpful diagrams and sketches.

It seems likely that many will heed Dr. Dalldorf's suggestion to hospital pathologists and bacteriologists that they employ these methods simply to improve diagnosis in afflictions of suspected virus etiology without the necessity of indulging in extensive original investigative work.

IVAN L. BENNETT, JR.

Surgery of the Ambulatory Patient, 3rd edition. By L. K. FERGUSON. 866 pp., $12.00. J. B. Lippincott Co., Philadelphia, Pa.

The text by Dr. Ferguson on surgery of the ambulatory patient (a far more descriptive title than the usual "Minor Surgery") has long been a classic in its field. The 3rd edition will be even more popular than the previous ones. The book is excellently illustrated, using a combination of photographs, line sketches, and half-tones. It is an encyclopedic type of book and the general practitioner, the surgeon, and the medical student will all find useful, practical information on a wide variety of conditions. As might be expected in such a work, there is some repetition since conditions are taken up both by pathologic and regional classification. There is a good index. In addition, an excellent bibliography enhances the text.

This reviewer does not always agree with every detail of treatment as stated in the text but the disagreements are not profound or basic. The section on Burns does not seem quite up to the general level of this book. The reviewer also wishes that the author had emphasized

the importance of biopsy and of pathologic examination of all tissue removed when dealing with cysts and tumors. The use of testosterone in the treatment of breast pain in young women seems also questionable. Finally, it is to be hoped that there will be correct spelling of the name Halsted in a subsequent edition.

The acquisition of this book is highly recommended to those whose office practice includes what is commonly called "minor surgery".

EDWARD S. STAFFORD

Wiederbelebung. By H. KILLIAN and A. DÖNHARDT. 320 pp., Ganzleinen DM 24. ($5.70). Georg Thieme Verlag, Stuttgart, Germany. Also Intercontinental Book Corp., New York, N. Y.

The problems of respiratory resuscitation are of great interest to the physiologist, to the student of medicine, and to anyone who may have to manage the patient in need of this vital form of assistance.

This excellent text by Killian and Dönhardt should be of special use to the student. It offers little that is new to the physiologist, and is too complex a presentation to be of practical use to those in need of simple advice on resuscitative methods.

Some of the suggestions, such as those regarding the use of analeptics and the warming of the injured are open to question.

In general it is an interesting and stimulating text on a much neglected subject.

DONALD F. PROCTOR

Premature Infants. A Manual for Physicians, 2nd edition. By ETHEL C. DUNHAM. 459 pp., $8.00. Paul B. Hoeber, Inc., New York, N. Y.

The first edition of this book published by the government in 1948 was one of the greatest bargains in medical books. Now published under private auspices it still is an exceptional bargain. Here one of the leading authorities in the field has collected, sifted and annotated all that is known about premature infants.

The first section deals with such general considerations as definitions, criteria, incidence, etiology, rates of development, and prognosis. The second section which deals with clinical considerations will be the most useful to nurses and physicians. Here emphasis is laid on proper evaluation of the newborn premature infant and upon safe conservative care. Detailed consideration is given to the diagnosis and therapy of all abnormal conditions which occur in the premature infant. The third section deals with the public health aspects of prematurity. The statistics of prematurity and programs for the care of premature infants are thoroughly discussed. A long series of appendices including various statistical data and methods for special study concludes the book.

The reader will be struck by the fact that relatively little space is given to the etiology and prevention of prematurity. This does not, however, reflect a fatal weakness in an otherwise excellent book. Rather it reflects our vast ignorance of the causes of prematurity, a matter of perhaps greater importance than those matters which have been so thoroughly considered in this volume. Until great advances are made in the physiology of uterine and placental action, the approach to prematurity exemplified by Dr. Dunham's book must remain of paramount importance.

GEORGE W. CORNER, JR.

Medicine for Nurses, 2nd edition. By M. TOOHEY. 640 pp., $6.50. E. & S. Livingstone, Ltd., Edinburgh and London, England. The Williams and Wilkins Co., Baltimore, Md.

Along with its discussions of medical diseases this book contains chapters on related subjects such as "Acute Infectious Fevers", "Important Drugs", as well as a chapter on "Psychological Medicine" written by Henry R Rollins, M.D., D.P.M.

In the chapters dealing with the medical diseases, there is excellent presentation of signs, symptoms, and treatment. However, nursing measures necessary in the care of the patient are not included in many of the areas. Diagnostic tests associated with the various disorders are discussed in detail.

The chapter entitled "Important Drugs" gives a brief summary of the more common drugs and their uses, but no attempt is made to explain their mode of action. Generally, the average range of dosage as well as the toxic manifestations are listed.

In his chapter on "Psychological Medicine" Dr. Rollins briefly discusses the psychological development of the individual, the neuroses, psychosomatic medicine, the psychological effects of illness and the psychological role of the nurse. While brief, this might aid the nurse who enters medical nursing without psychiatric experience.

GERALDINE JORDAN

Administrative Medicine. Transactions of the Third Conference, October 6, 7 and 8, 1954. Edited by GEORGE E. STEVENSON. 172 pp., $3.00. The Josiah Macy, Jr. Foundation, New York, N. Y.

This volume, published by the Josiah Macy, Jr. Foundation, consists of edited verbatim transactions of a conference on Administrative Medicine held October 6, 7, and 8, 1954. The conference was attended by over twenty outstanding individuals in the fields of Medicine, Public Health, Hospital Administration, Public Administration and faculties in Sociology.

The volume is concise and clearly written and presents discussions on a broad concept of Administrative Medicine of which Hospital Administration, Prepayment Plan Administration, Public Health Administration, to name a few, are parts. Individuals active in any administrative work in the fields of Health and Medicine will find this volume informative and very stimulating.

RUSSELL A. NELSON

Clinical Biochemistry, 5th edition. By ABRAHAM CANTAROW and MAX TRUMPER. 738 pp., $9.00 W. B. Saunders Co., Philadelphia, Pa.

The application of knowledge in the field of physiological chemistry to problems of disease and to evaluation of disease processes offers challenging and growing possibilities to the physician and the biochemist, alike. In this developing field an increasing number of textbooks are appearing of which "Clinical Biochemistry" is one. Some of the other texts are: (1) "Biochemistry in Relation to Medicine," by Carter and Thompson from Queens College and Guy's Hospital, 2nd ed., 1953; (2) "Biochemistry and Human Metabolism," by Walker, Boyd, and Asimov from Boston University, 2nd ed., 1954; (3) "The Biochemistry of Clinical Medicine," by Hoffman from the University of Illinois College of Medicine, 1954; (4) "Human Biochemistry," by Kleiner from the New York Medical College, 4th ed., 1954.

The book by Cantarow and Trumper has to meet the same difficulty that the others attempt to meet, namely, the overwhelming vastness of the basic and clinical material to be covered. Like its predecessors it is largely another textbook of biochemistry. On the one hand wide coverage and basic theory and background in the field of biochemistry are and must be curtailed to make room for the brief and limited excursions into some facets of disease on the other. The present authors make a valiant effort to provide a useful bridging of these domains but no one has yet performed this Herculean task to any great degree.

The book is useful for a degree of beginning orientation on certain subjects for either physician or biochemist. For each it provides a practical and fairly concentrated survey of some of the other's knowledge and this can be very helpful. It is quite readable. It includes

an unusual amount of material on disease for this type of book and is probably useful in the reference shelf of the physician. In general it appears to be accurate except for the necessary restriction of meaning where need for brevity prevails.

F. W. BARNES, JR.

BOOKS RECEIVED FOR REVIEW

Atopic Dermatitis. By RUDOLF L. BAER. 112 pp., $2.50. J. B. Lippincott Co., Philadelphia, Pa.

The Brazen Serpent. By CHESNEY RAMAGE. 188 pp., $3.00. Vantage Press, New York, N. Y.

Die Überfunktion der Nebennieren. By RUDOLF CHWALLA 330 pp., $12.00. Wilhelm Maudrich, Vienna, Austria. Also Intercontinental Medical Book Corp., New York, N. Y.

The Dispensatory of the United States of America, 25th edition. Edited by ARTHUR OSOL and GEORGE E. FARRAR. 2139 pp., $25.00. J. B. Lippincott Co., Philadelphia, Pa.

District Nursing, 2nd edition. By ELEANOR JEANETTE MERRY and IRIS DUNDAS IRVEN. 262 pp., $4.50. Bailliere Tindall and Cox, London, England. Distributed by The Williams & Wilkins Co., Baltimore, Md

Medical Support of the Army Air Forces in World War II. By MAE MILLS LINK and HUBERT A. COLEMAN. 1027 pp., $7.00. U. S. Government Printing Office, Washington 25, D. C.

Of Research People. By GEORGE E. BURCH. 56 pp., $3.00. Grune & Stratton, New York, N. Y.

Peripheral Vascular Disease. By A. J. BARNETT and J. R. E. FRASER. 219 pp., $9.50. Melbourne University Press, Melbourne, Australia. Also Cambridge University Press, New York, N. Y.

Praktische Mikroskopie. By EWALD SCHILD. 242 pp., $6.00. Wilhelm Maudrich, Vienna, Austria. Also Intercontinental Medical Book Corp., New York, N. Y.

The Relief of Symptoms. By WALTER MODELL. 450 pp., $8.00. W. B. Saunders Co., Philadelphia, Pa.

Symposium on Genetic Recombination. A Reprint from the Journal of Cellular and Comparative Physiology, Vol. 45, Supp. 2, May, 1955. Edited by ALEXANDER HOLLAENDER and sponsored by The Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee.